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# The role of the cohesin loader in genome stability: a journey from yeast to human

Fosco Giordano

Karolinska Institutet

From Department of Cell and Molecular Biology
Karolinska Institutet, Stockholm, Sweden

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By

**Fosco Giordano**

*Principal Supervisor:*
Docent Lena Ström
Karolinska Institutet
Dep. of Cell and Molecular Biology

*Co-supervisor(s):*
Professor Piergiorgio Percipalle
NYU, Abu Dhabi

PhD Christopher Bot
Karolinska Institutet
Dep. of Cell and Molecular Biology

*Opponent:*
Professor Jessica Downs
University of Sussex
Genome Damage and Stability Centre

*Examination Board:*
Professor Stefan Åström
Stockholm University
Dep. of Molecular Biosciences

Docent Teresa Frisan
Karolinska Institutet
Dep. of Cell and Molecular Biology

Docent Herwig Schüler
Karolinska Institutet
Dep. of Medical Biochemistry and Biophysics

*To Giulia*

# ABSTRACT

Structural Maintenance of Chromosome (SMC) complexes, as their name suggests, have a central role in maintaining the higher structure of genomes, from bacteria to human, and in doing so protecting their integrity.

Cohesin, one of three SMC complexes, is required to hold sister chromatids together until anaphase, and for homologous recombination-based DNA repair. In these cellular processes, a separate complex, named NIPBL/MAU2 (Scc2/4 in *Saccharmomyces cerevisiae*) is needed to drive the loading of cohesin onto DNA.

This thesis focuses on the cohesin loader, in different model organisms and in the different cellular functions in which $NIPBL^{Scc2}$ is involved.

**Paper I** describes the requirements for Scc2 binding at an HO-induced DNA double strand break. ChIP-qPCR profiles show presence of Scc2 after break induction 30 kb around the break with strong binding 5 kb from the HO cut-site. Moreover, these Scc2 levels are found to depend on the MRX complex, the Tel1 kinase and H2A phosphorylation, but unlike cohesin not on Mec1.

Conversely **Paper II**, performed in human cell lines, shows a dual recruitment model for NIPBL at laser and FokI endonuclease-induced DNA damage. First, NIPBL is recruited to DSB via an HP1 binding motif located in its N-terminal. On the contrary NIPBL truncations containing the HEAT repeat rich C-terminal region, but lacking the HP1 motif, are not recruited at FokI foci but localizes only at laser tracks. The latter pathway depends on the activity of ATR/ATM kinases. Moreover a role for the ubiquitin ligases RNF8/RNF168 in the NIPBL recruitment to DNA damage is also described.

In recent years a new function was discovered, for cohesin and its loader, in gene regulation. **Paper III** shows that Scc2 affects both general gene expression and DNA damage dependent transcription by microarray analysis. Lastly **paper IV** focuses on another important process in which cohesin is involved, meiosis, describing NIPBL chromosomal localization in male and female murine germ cells, during meiotic prophase I.

# LIST OF SCIENTIFIC PAPERS

I. GIORDANO F., Rutishauser D., and Ström L. Requirements for DNA double strand break accumulation of Scc2, Similarities and Differences with Cohesin. *Manuscript.*

II. Bot C., Pfeiffer A., GIORDANO F., Edara D. M., Dantuma N. P. and Ström L. Independent Mechanisms Recruit the Cohesin Loader Protein NIPBL to Sites of DNA Damage. *Manuscript.*

III. Lindgren E., Hägg S., GIORDANO F., Björkegren J., and Ström L. Inactivation of the budding yeast cohesin loader Scc2 alters gene expression both globally and in response to a single DNA double strand break. *Cell cycle*, 2014, 12, 3645-58.

IV. Visnes T., GIORDANO F., Kuznetsova A., Suja J. A., Lander A., Anne L Calof and Lena Ström. Localisation of the SMC loading complex Nipbl/Mau2 during mammalian meiotic prophase I. *Chromosoma,* 2014, 123, 239-52.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 53BP1 | p53 binding protein 1 |
| ATM | Ataxia telangiectasia mutated |
| ATR | Ataxia telangiectasia and Rad3-related |
| bp/kb | base pair/ kilobase pair |
| BRCA 1,2 | Breast cancer antigen 1,2 |
| BrdU | Bromodeoxyuridine |
| CAR | Cohesin associated region |
| CdLS | Cornelia de Lange syndrome |
| ChIP | Chromatin immunoprecipitation |
| CTCF | CCCTC-binding factor required for transcriptional regulation |
| CHK1,2 | Checkpoint kinase 1,2 |
| CDK | Cyclin-dependent kinase |
| CSD | chromoshadow domain |
| DI-Cohesion | Damage-induced cohesion |
| DNA | Deoxyribonucleic acid |
| DNA lig4 | DNA ligase 4 |
| DNA PKcs | DNA dependent protein kinase catalytic subunit |
| Dnl4 | DNA ligase 4 |
| DSB | double strand break |
| Eco1 | Establishment of cohesion 1 |
| ESCO1,2 | Establishment of cohesion 1,2 |
| FACS | Fluorescence-activated cell sorting |
| HEAT | Huntingtin, Elongation factor 3, protein phosphatase 2A and Tor1 |
| H2A(X) | Histone 2A(X) |
| HR | Homologous recombination |

| | |
|---|---|
| Lif1 | Ligase interacting factor 1 |
| Mec1 | Mitosis entry checkpoint 1 |
| Mre11 | Meiotic recombination 11 homolog A |
| MRN/MRX | MRE11 RAD50 NBS1/Mre11 Rad50 Xrs2 |
| NBS1 | Nijmegen breakage syndrome defective 1 |
| Nej1 | Nonhomologous end joining defective 1 |
| NHEJ | Nonhomologous end joining |
| NIPBL | Nipped-B-like |
| ORF | open reading frame |
| PCNA | Proliferating cell nuclear antigen |
| PDS5 | Precocious dissociation of sisters 5 |
| PFGE | Pulse-field gel electrophoresis |
| PLK1 | Polo-like kinase 1 |
| qPCR | Quantitative polymerase chain reaction |
| RAD | Radiation sensitive |
| RSC | Remodel the Structure of Chromatin |
| SA | Stromal antigen |
| Sae2 | Sumo activating enzyme subunit 2 |
| SCC | Sister chromatid cohesion |
| Sgs1 | Small growth suppressor 1 |
| SMC | Structural maintenance of chromosome |
| ssDNA | single strand DNA |
| Tel1 | Telomere maintenance 1 |
| TMP | trimethylpsoralen |
| TRP | Tetratricopeptide |
| WAPL | Wings apart like |
| XLF | XRCC4-like factor |

| | |
|---|---|
| XRCC4 | X-ray repair cross-complementing protein 4 |
| Xrs2 | X-ray sensitivity 2 |

# 1 INTRODUCTION

## 1.1. GENOME STABILITY

Genome stability is the sum of processes that a cell employs to preserve and to deliver free of error to daughter cells, its genetic information; it is a broad concept including events connected to DNA replication, maintenance of chromosome structure during the cell cycle, and DNA repair.

Orthologs important for genome integrity usually exert the same function, but might carry different names. To avoid confusion I will refer to the metazoan gene or protein, putting the *Saccharomyces cerevisiae* version in superscript (i.e. $MFG^{Mfg}$). In case a protein function is unique for a certain organism, only the name of that specific protein or gene will be used.

## 1.2. THE CELL CYCLE

The cell cycle represents all the steps required for a single cell to grow, replicate and propagate DNA, in order to generate two daughter cells with identical genetic information.

A cell cycle is composed of four different phases: a DNA replication phase called S, in which the genetic material is duplicated, generating two DNA molecules called sister chromatids, and a cell division phase called M, which comprises two major events: nuclear division or Mitosis, and cytokinesis. M phase is composed of sub-phases when DNA is structured and reorganized inside the cell; in *prophase* the genetic material is condensed in rod-like structures kept together by sister chromatid cohesion. Subsequently, during *metaphase,* DNA is attached to a microtubule-based structure, called the spindle, and aligned at the center of the cell. During *anaphase,* sister chromatids separate and migrate to the opposite poles of the cell. In the last portion of mitosis (*telophase*) before cytokinesis, the spindle is disassembled and DNA is de-condensed into new nuclei (Figure 1).

Between the S and M phases there are two gap phases (G1 and G2) that are needed for cells to grow, double their mass, produce new organelles and monitor if environmental and internal conditions are suitable for DNA replication and cell division.

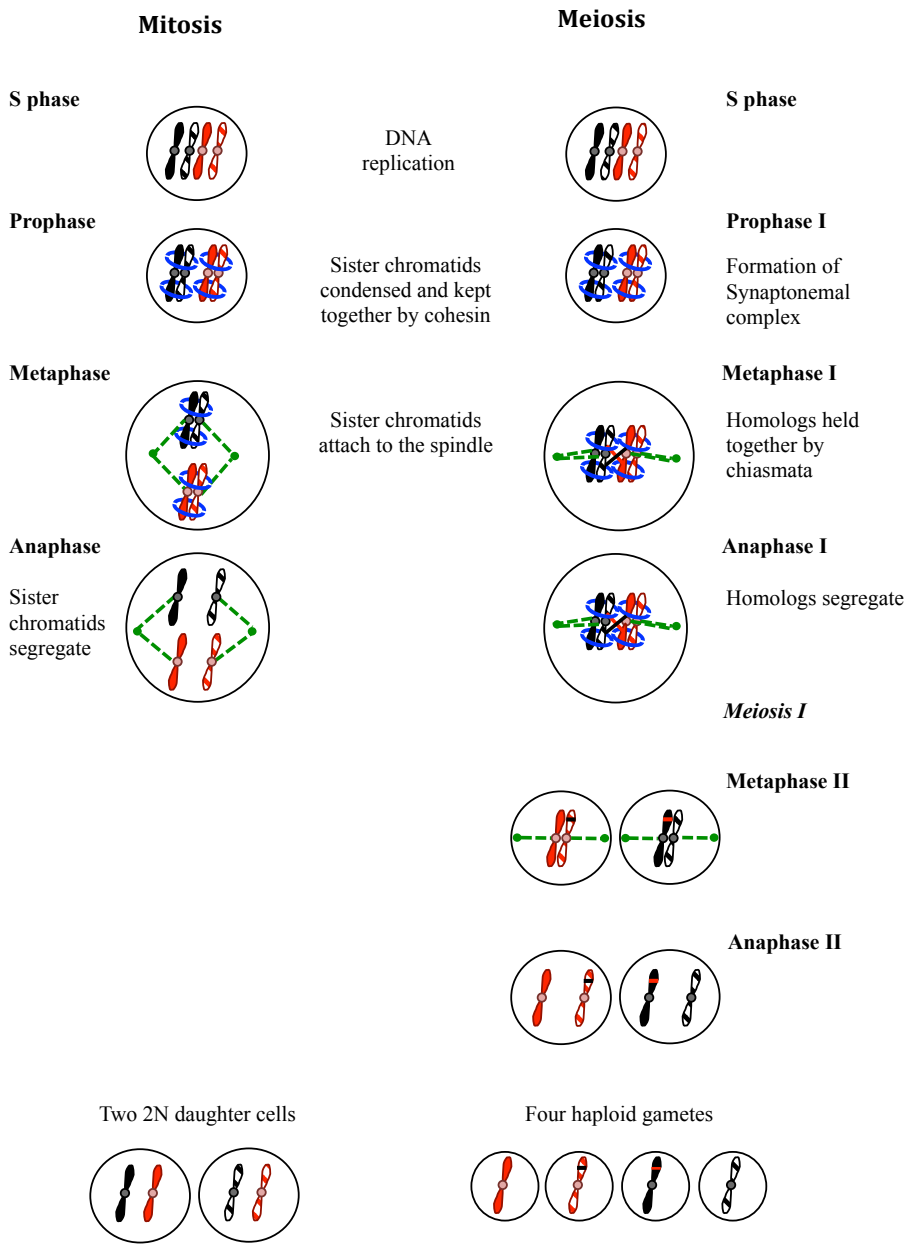


Figure 1: Scheme of the different phases of mitosis (on the left) and meiosis (on the right). Blue rings around chromosomes represent cohesin molecules. In black and red are represented maternal and paternal chromosomes respectively.

## 1.3. MEIOSIS

Meiosis is a specialized form of nuclear division that occurs in diploid eukaryotes reproducing sexually, leading to the formation of four haploid cells, which then differentiate into reproductive cells called gametes. Meiosis starts with DNA replication, meiotic S phase, followed by two consecutive cell divisions called meiosis I and meiosis II (Figure 1). After meiotic S phase chromosomes are present as two pairs of sister chromatids, called homologs, connected through non-sister linkages.

To help homolog pairing and facilitate the resolution of DSBs, a protein structure called synaptonemal complex (SC) is formed. The appearance of the SC changes through prophase I and defines four different sub-phases: *leptotene*, *zygotene*, *pachytene* and *diplotene*. Right after replication, during *leptotene*, axial elements (AE) composed of SYCP2 and SYCP3 are formed. During *zygotene,* when the homologs start to pair, transverse filaments (TF), composed of SYCP1, are loaded between the AEs, forming the central element (CE). During *pachytene*, the homologs are aligned and tied together along their entire length by the SC in a process called synapsis. After *pachytene* the AE starts to dissociate, a process that ends during *diplotene*.

Resolution of the SC is tightly regulated, such that the linkage it forms between the non-sister chromatids remains until DNA exchange between homologs, also called crossovers, have been established. The homologs are kept together by chiasmata, the visible crossing overs between chromosomes formed thanks to homologous recombination based DNA repair of double strand breaks (DSB). During meiosis II, sister chromatids from each homolog are then separated in essence through conventional mitosis (Handel, 2010).

## 1.4. DNA REPAIR

Cells are continuously under the risk of encountering DNA damage, from environmental sources such as chemicals and ionizing radiation, or cellular processes like oxidative stress or replication fork collapse. A number of mechanisms protect the genetic material from harmful events, in form of mutations, deletions or rearrangements that can ultimately lead to cell death.

### 1.4.1. Early events in DNA damage repair

The initial steps in DNA damage repair include: recognition of the damage, checkpoint activation and modification of DNA ends at the break. The metazoan MRN (MRE11,

RAD50, NBS1) (De Jager, 2001) or yeast MRX (Mre11, Rad50, Xrs2) (Lisby, 2004) complex, together with, but independently of KU70/KU80$^{Ku70/Ku80}$, are recruited to the site of DNA damage early (Milne, 1996). These two complexes affect the choice of repair pathway: Homologous Recombination (HR) via MRN$^{MRX}$ or Non-Homologous End Joining (NHEJ) through KU70/KU80$^{Ku70/Ku80}$. The selection of one of the two mutually exclusive repair mechanisms mostly depends on the cell cycle phase in which the damage took place. After DNA replication, HR becomes not only available but also a favored choice, especially in budding yeast, where the cyclin dependent kinase (CDK) promotes the switching from NHEJ to HR (Aylon, 2004). Evidence indicates in fact an increased expression of HR factors after S phase (Chen, 1997). In mammalian cells however, NHEJ is the mostly used pathway. Even in G2, 80% of the cells still repair DNA damage via NHEJ (Beucher, 2009; Shibata, 2011).

In human, MRN recruits the ATM kinase for HR (You, 2005) while KU70/KU80 recruits the DNA-PKcs kinase for NHEJ (Gottlieb, 1993). In budding yeast on the other hand, the ATM ortholog Tel1 is recruited by the MRX complex (Nakada, 2003) and its activity is necessary for both HR and for NHEJ (X. Zhang, 2005). Tel1 phosphorylates the histone H2A (Redon, 2003), while both ATM and DNA-PKcs are capable of post-translationally modify H2AX, the mammals H2A histone variant (Rogakou, 1998, 1999; Stiff, 2004). Phosphorylation of H2AX$^{H2A}$ spreads from the break and promotes the recruitment of additional factors for DNA repair (Paull, 2000).

In case a cell is not capable of a rapid and efficient response to DNA lesions in order to ensure enough time for proper repair, the cell cycle is arrested by activation of a DNA damage checkpoint. Three different DNA damage checkpoints are available; the G1, the intra-S, and the G2/M phase checkpoints ( Paulovich, 1995; Siede, 1996; Weinert, 1988).

### 1.4.2. Homologous recombination

As mentioned before, in case an undamaged DNA template is available, cells can repair DNA DSBs by HR (Figure 2 and refer to Table 1 for a list of factors involved in HR in human and yeast).

Cells commit to HR when DNA ends are subjected to initial 5´- 3´end-resection by the MRN$^{MRX}$ complex and the endonuclease CtIP$^{Sae2}$ (Clerici, 2005; Sartori, 2007), creating ssDNA, which becomes substrate for long-range resection by EXO1$^{Exo1}$ or BLM2/DNA2$^{Sgs1/Dna2}$ (Mimitou, 2008; Nimonkar, 2011). Formation of ssDNA also marks the

initiation of dissociation of CtIP$^{Sae2}$, ATM$^{Tel1}$, and MRX (while MRN stays on DNA), and consequent binding of RPA$^{RPA}$ to DNA ends which prevents their degradation.

RPA$^{RPA}$ is also necessary for the recruitment of ATR$^{Mec1}$ through the regulatory subunit ATRIP$^{Ddc2}$ (Zou, 2003), and of Rad52 (yeast) or BRCA2 (metazoans) which mediate substitution of RPA with RAD51$^{Rad51}$ (New, 1998). RAD51$^{Rad51}$ is the recombinase that catalyze the formation of a D loop by mediating the strand invasion of one of the ssDNA ends, followed by replication of 3´DNA ends (Shinohara, 1992). An additional factor is the chromatin remodelling ATPase RAD54$^{Rad54}$ that stimulates RAD51$^{Rad51}$ binding to DNA and the formation of the D-loop (Clever, 1997; Swagemakers, 1998; Wolner, 2005). In yeast Rad59 facilitates Rad52 binding at break sites (Davis, 2001).

The final step of DSB repair is the formation of a Holliday junction (HJ), created by the annealing of the remaining 3´ end with the opposite broken strand. Resolution of the HJ can lead to a product, with or without cross-over, depending on the resolution method.

Until now this model of repair is the most accepted and it is often used to explain meiotic DSB recombination, on the other hand mitotic recombination has a lower level of cross-over events. To explain this phenomenon two other models were formulated: the synthesis dependent strand annealing (SDSA) and the migrating D-loop models, which are normally referred both as SDSA.

The first one proposes that, contrary to the DSB repair model, both 3´ends invade the homologous strands, however after limited DNA synthesis both strands are displaced and anneal the complementary 5´ strands followed by fill-in that results in repair with a non cross-over product. The migrating D-loop model on the other hand proposes, similarly to the DSB repair model, that a single 3´end invades the homologous duplex. A limited DNA synthesis provides the sufficient template for repair, and the strand is then displaced and anneal to the other 3´end. Again the consequent fill-in produces a non-crossover product (Symington, 2014).

| Role in DSB repair | *S. cerevisiae* | *H. Sapiens* |
|---|---|---|
| End resection | Mre11-Rad50-Xrs2 | MRE11-RAD50-NBS1 |
| | Sae2, Exo1 | CtIP, EXO1 |
| | Dna2-Sgs1 | DNA2-BLM |
| Adaptors | Rad9 | 53BP1, |
| | - | MDC1 –BRCA1 |
| Checkpoint Signaling | Tel1 | ATM |
| | Mec1-Ddc2 | ATR-ATRIP |
| Single-strand DNA coating | Rfa1 – Rfa2- Rfa3 (RPA) | RPA1 – RPA2 – RPA3 (RPA) |
| Single-strand annealing | Rad52 | RAD52 |
| | Rad59 | - |
| Mediators | - | BRCA2 |
| | Rad52 | - |
| Strand invasion | Rad51 | RAD51 |
| | Rad54 | RAD54A, RAD54B |

Table 1: List of different factors involved in HR, classified according to their functions in DSB repair, in budding yeast and corresponding orthologs in human.

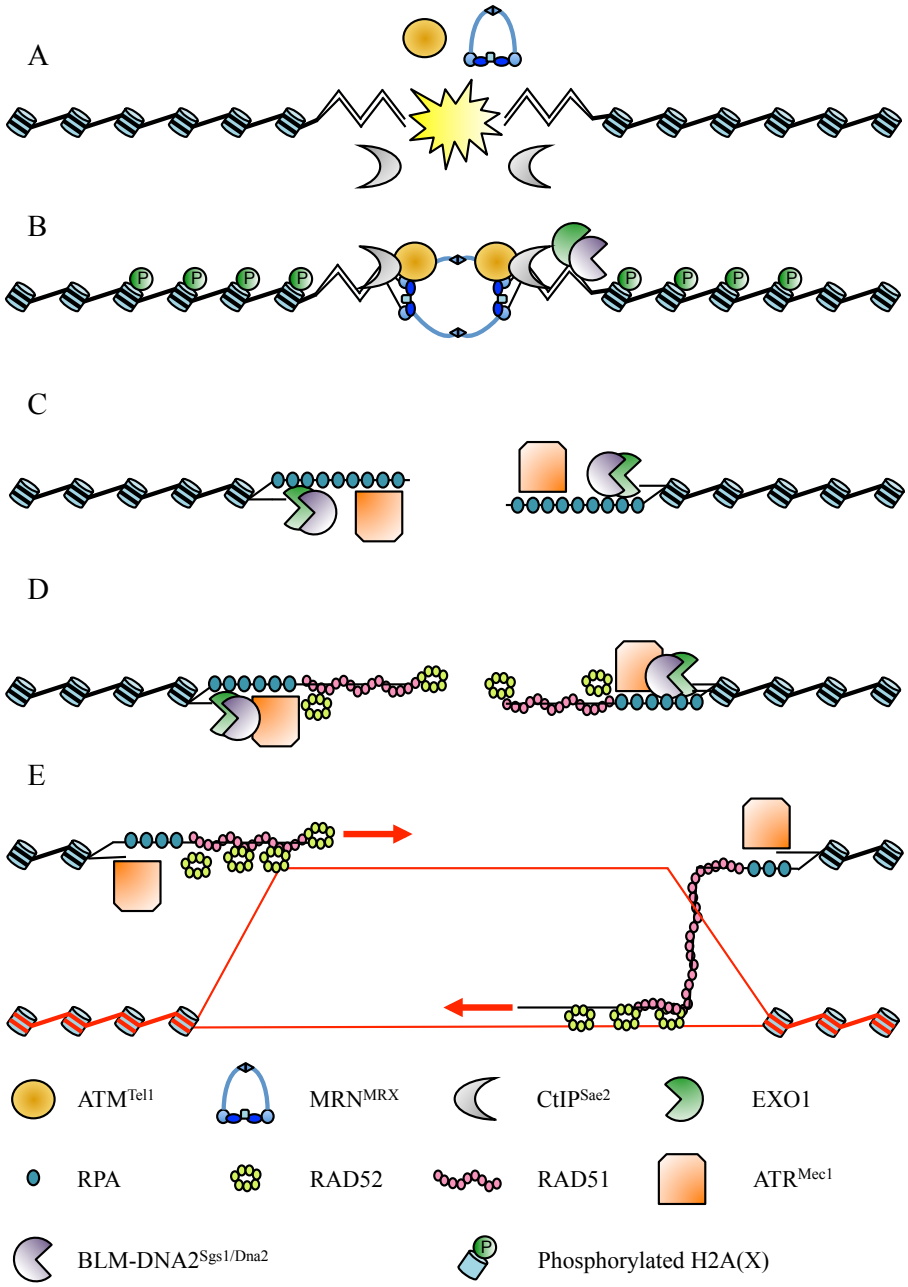


Figure 2: Scheme of Homologous Recombination: (A) DNA damage, recruitment of $MRN^{MRX}$ complex, $ATM^{Tel1}$ and $CtIP^{Sae2}$ (B) H2A(X) phosphorylation and short range resection by $MRN^{MRX}$ and $CtIP^{Sae2}$. (C) EXO1 and BLM-$DNA2^{Sgs1/Dna2}$ depedent long-range resection; RPA and $ATR^{Mec1}$ recruitment. (D) Recruitment of RAD52 and RAD51. (E) Formation of a D loop followed by creation of a Holliday junction and repair.

### 1.4.3. Non Homologous End Joining

Classical NHEJ is the process of ligation of DNA ends at a DSB, and considered a rapid pathway for the cell to deal with the repair of the same. However, it is also considered an error prone mechanism. In fact DNA ends need to be processed if they are not compatible for ligation, thus the loss of short sequences is quite common (Lieber, 2010). Additional factors of NHEJ are recruited by the initial binding of the Ku complex; first the DNA end processing complex Artemis-DNA-PKcs binds KU70/80 (in budding yeast Mec1 or Tel1 substitute for PKcs), then the break is repaired by DNA ligase IV together with its cofactors $XRCC4^{Dnl4}$ and $XLF^{Lif1}$, assisted by Nej1 in yeast (Lieber, 2010).

### 1.4.4. Other events in DNA damage repair

Aside from the actual recognition of DNA damage and joining of the broken ends, additional events are essential in order to ensure proper repair.

One is modification of chromatin; $H2AX^{H2A}$ phosphorylation was previously mentioned as one of the most important modifications related to DNA damage. Both histone bodies and tails are however subjected to multiple post-translational modifications (phosphorylation, acetylation, ubiquitination, sumoylation and methylation). The role of these modifications is to recruit factors at different stages of the repair process. Of the many examples that can be described, relevant for this thesis, is MDC1, which by sensing ubiquitinated histone H1 (Thorslund, 2015), recruits RNF8 that in turn recruits RNF168. H2A and H2AX are then poly-ubiquitinated by the RNF168 E3 ubiquitin ligase, which in turn promotes the recruitment of other repair factors, such as 53BP1 and BRCA1 (Doil, 2009; Huen, 2007; Kolas, 2007; Stewart, 2009).

Another event in DNA damage is change of transcription of two classes of genes. The first one is composed of transcripts whose products are directly involved in the repair process. The second class includes genes encoding proteins related to DNA metabolism. The specific genes whose expression is changed depends on the type of DNA damage.

The change in gene expression due to DNA damage requires a complex transduction pathway. The loss of DNA integrity activates various sensors, depending on the type of lesion and cell cycle phase. The signal derived from the sensors is amplified by the transducers, often kinases, and relayed to effectors. These are likely transcription factors acting on the promoters of the target genes (Fu, 2008).

In order to respond to a threat that can impair cell survival, the expression of multiple other genes is either induced or repressed in a mechanism called environmental stress response (ESR). Repressed genes are involved in protein synthesis, likely in order for the cell to preserve energy. Induced genes on the other hand are related to cellular functions spanning from oxidation-reduction, maintenance of protein stability and balancing of osmolarity (Gasch, 2001, 2002).

## 1.5. SMC COMPLEXES

SMC (structural maintenance of chromosome) proteins are a conserved family of proteins, present from bacteria to humans and with central roles in regulating genome stability by maintaining chromosome structure during mitosis and meiosis, and having additional functions in gene regulation and DNA repair.

SMC proteins are characterized by a typical structure; two nucleotide binding motifs, named Walker A and Walker B, are located at the N- and C- terminals respectively. The two protein ends interact, forming the HEAD domain, thanks to an anti-parallel folding of the peptide chain into a structure called coiled-coil motif. Opposite to the HEAD domain is the HINGE, through which two SMC monomers interact with each other (Figure 3) (M. Hirano, 2002; Melby, 1998).

While bacteria contain only one homodimeric SMC complex (Melby, 1998), eukaryotes possess three heterodimeric complexes, composed of six different SMC proteins. Cohesin, formed by SMC1 and SMC3, is involved in sister chromatid cohesion and DNA repair, Condensin (SMC2 and SMC4) mainly promotes DNA condensation, and the SMC5/6 complex has been suggested to resolve DNA topological structures derived from DNA replication stress and is also involved in DNA repair (Guacci, 1997; T. Hirano, 1994, 1997; Kegel, 2011; Lehmann, 1995; Michaelis, 1997).

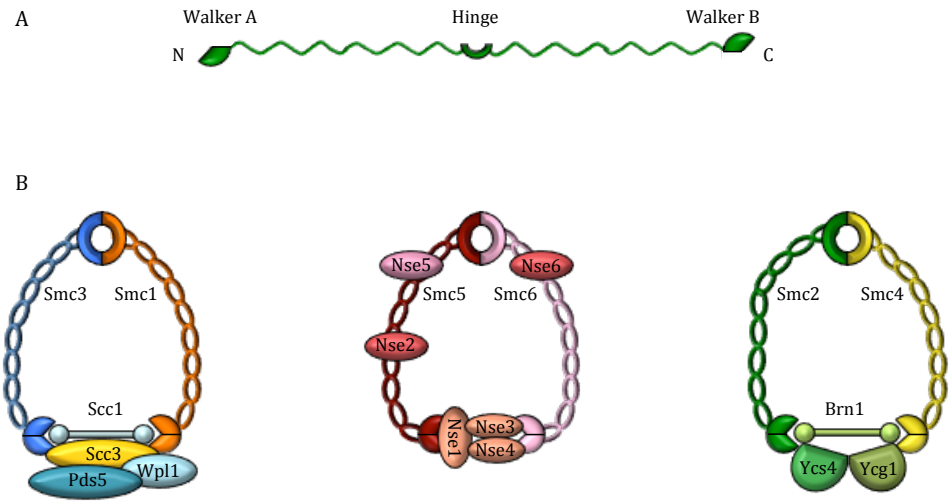


Figure 3: Representation of the different domains in an unfolded SMC protein. The N- and C-terminals interact due to the protein folding at the Hinge domain (A). The three *S. cerevisiae* SMC complexes with core and accessory proteins (B).

### 1.5.1. The cohesin complex

Cohesin is a multi subunit complex composed of, in addition to the two already mentioned SMC proteins SMC1$^{Smc1}$ and SMC3$^{Smc3}$, RAD21$^{Scc1}$, a member of the kleisin family and either SA1 or SA2 in metazoans, orthologs of yeast Scc3 (Table 2).

RAD21$^{Scc1}$ binds the HEAD domains of SMC1$^{Smc1}$ with its C-terminus, and SMC3$^{Smc3}$ with its N-terminal portion, creating a tripartite ring (Haering, 2002, 2008). As for the other SMC complexes (Figure 3), accessory proteins are associated with cohesin. These are called PDS5$^{Pds5}$ and WAPL$^{wpl1}$ and interact both with each other, and with the large HEAT repeat protein SA1/2$^{Scc3}$. Moreover, PDS5$^{Pds5}$ interacts with cohesin via Rad21$^{Scc1}$ (Hartman, 2000; Kueng, 2006; Panizza, 2000). However, Wpl1 does not bind cohesin in a stoichiometric manner, in fact only some cohesin complexes contain Wpl1 (Chan, 2012). Associated with cohesin in vertebrates is an additional component called sororin. Unlike other cohesin accessory proteins its binding appears to be cell cycle dependent (Nishiyama, 2010; Rankin, 2005; Schmitz, 2007). To accommodate DNA the cohesin "ring" needs to be opened, and the proposed "entry gate" is situated between the hinges of SMC1$^{Smc1}$ and SMC3$^{Smc3}$ (Gruber, 2006) (Figure 3). Cohesin can also be removed from chromosomes, not only through Rad21$^{Scc1}$ degradation (further discussed below), but also via an exit gate located between SMC3$^{Smc3}$ and Rad21$^{Scc1}$ (Buheitel, 2013; Chan, 2012).

| Function | *S. cerevisiae* | *H. Sapiens/M. musculus* |
|---|---|---|
| Cohesion | Smc3 | SMC3 |
| | Smc1 | SMC1α (SMC1β) |
| | Scc1 (Rec8) | RAD21, (RAD21L, REC8) |
| | Scc3 | SA1, SA2 (SA3) |
| Loading | Scc2 | NIPBL |
| | Scc4 | MAU2 |
| Establishment | Eco1 | ESCO1, ESCO2 |
| Maintenance | Pds5 | PDS5A, PDS5B |
| | Wpl1 | WAPL |
| | - | Sororin |
| Dissolution | Esp1 | Separase |
| | Pds1 | Securin |

Table 2: List of cohesin subunits and accessory proteins in budding yeast and corresponding orthologs in human, divided on function. In brackets meiosis specific subunits.

### 1.5.2. The cohesin loader

Cohesin is loaded onto DNA by a separate complex, an heterodimer first discovered in budding yeast (Ciosk, 2000), but present in all eukaryotes investigated (Gillespie, 2004; Krantz, 2004; Rollins, 2004; Seitan, 2006; Takahashi, 2004; Tonkin, 2004; Watrin, 2006). The *Saccharomyces cerevisiae* cohesin loader Scc2/4, and the human NIPBL/MAU2, share a certain degree of similarity. $NIPBL^{Scc2}$ is a large HEAT repeat protein (Neuwald, 2000) while $MAU2^{Scc4}$ is a tetratricopeptide repeat (TRP) protein; two different kinds of repeats with a common feature of protein-protein interaction. However unlike cohesin, the protein sequence of both the subunits of the loader are poorly conserved between yeast and metazoan.

It is not clear how the loader exerts its function, previous reports have shown that the ATPase activity of cohesin is important for its DNA association (Arumugam, 2003) and *in vitro* studies on *Schizosaccharomyces pombe* Mis4$^{Scc2}$ have shown that the loader affects cohesin ATP hydrolysis (Arumugam, 2003; Murayama, 2014). Moreover it appears that the HEAT repeats are necessary for Scc2 recruitment of cohesin (Takahashi, 2008).

Little is known about the structure of NIPBL$^{Scc2}$, neither which region of the protein is required for DNA binding, nor what domains are involved in cohesin interaction. Work in *Xenopus laevi* however shows that the first 500 aminoacids of NIPBL bound to MAU2 are capable of binding DNA (Takahashi, 2008). This could mean that the N-terminal of NIPBL is sufficient for DNA interaction, or MAU2 is, or a combination of the two.

Still *in vitro* studies on MAU2$^{Scc4}$ have shown that it is not required for the binding of Scc2 to naked DNA, but has been hypothesized to be necessary for *in vivo* chromatin interactions (Murayama, 2014). Recently, two independent studies have managed to obtain crystals of Scc4, which appears organized in three different domains, forming a hydrophobic channel that wraps the unstructured N-terminal of Scc2, in an anti-parallel orientation. A conserved patch on the surface of Scc4 is required for the recruitment of the loading complex at centromere regions *in vivo* (Chao, 2015; Hinshaw, 2015).

The human ortholog of Scc2 was named Nipped-B Like (NIPBL) after the *Drosophila melanogaster* version of the cohesin loader Nipped-B (Rollins, 2004), and was discovered as one of the causes of a developmental disorder called Cornelia de Lange Syndrome (Krantz, 2004; Tonkin, 2004). NIPBL is more than twice the size of the budding yeast version, thus it is possible to imagine that the metazoan Scc2 ortholog likely possesses new functions or forms of regulation not present in *S. cerevisiae*. One example is related to the fact that two different transcripts of NIPBL have been observed, encoding for two protein isoforms, NIPBL A and NIPBL B (Tonkin, 2004). To date no specific function has been associated to either splice variant. An other example can be the NIPBL PxVxL motif known to bind the chromoshadow domain (CSD) of HP1, a protein not present in budding yeast, which is known to interact with methylated histone H3, and be involved in gene silencing (Lechner, 2005).

## 1.6. THE COHESIN CYCLE IN BUDDING YEAST

### 1.6.1. G1 cohesin loading and localization

In budding yeast cohesin is loaded onto DNA from late G1 phase, by the cohesin loader (Ciosk, 2000). Multiple studies show an accumulation of the complex around centromeres and in pericentromeric regions (Glynn, 2004; Lengronne, 2004; Weber, 2004). On chromosomes arms cohesin localizes at AT rich regions, mostly in intergenic regions with converging transcription, even though no DNA binding motif has been linked to the complex (Lengronne, 2004).

The Scc2/4 complex is also enriched at centromeres, and this binding requires the kinetochore Ctf19 subcomplex (Eckert, 2007; Fernius, 2009; Ng, 2009). On chromosome arms however, it does not co-localize with cohesin but resides mostly at sites of high transcription (Hu, 2011; Lengronne, 2004). Due to this, a model has been proposed according to which cohesin is shifted to its terminal binding sites by the transcription machinery, and the loading and translocation are ATP dependent events that require the ATPase function of cohesin (Arumugam, 2003; Hu, 2011).

It appears that Scc1 expression is necessary to trigger Scc2/4 DNA binding, at least at centromeres where cohesin and the loader co-localize, as demonstrated by ectopic expression of Scc1 in early G1 phase (Fernius, 2013).

Furthermore, a recent study has proposed that the nucleosome remodeling complex RSC participates in cohesin loading by recruiting Scc2/4 to nucleosome-free regions (Lopez-Serra, 2014).

### 1.6.2. S phase and cohesion establishment

As previously mentioned cohesin loading is a dynamic event where the complex keeps dissociating from DNA. For its primary function, to maintain the sister chromatids paired until cell division, that was first discovered in *Saccharomyces cerevisiae*, loading is however not sufficient (Guacci, 1997; Michaelis, 1997). In order to keep sister chromatids together until anaphase, cohesin molecules must become cohesive, a process that requires acetylation on residues K112/113 of Smc3 by the acetyltransferase Eco1 (Ivanov, 2002; Rolef Ben-Shahar, 2008; J. Zhang, 2008). These modifications counteract the anti-establishment activity of Wpl1, Pds5 and Scc3 (Chan, 2012; Lopez-Serra, 2013; Rowland, 2009). Once cohesion has been established the Scc2/4 function becomes dispensable for cohesion maintenance (Ciosk, 2000).

Early studies have shown a dependency between cohesion and DNA replication (Skibbens, 1999; Tóth, 1999; Uhlmann, 1998). This was strengthened by the fact that Eco1 contains a PCNA-binding motif (Lengronne, 2006; Moldovan, 2006), DNA replication affects Eco1 acetyltransferase activity (Rolef Ben-Shahar, 2008) and multiple replication factors are required for sister chromatid cohesion (Sherwood, 2010). Two models were proposed to explain the basis for sister chromatid cohesion. The first one is the so-called "ring model" where a single cohesin molecule is capable of embracing two sisters. This hypothesis is supported by the fact that the kleisin subunit closes the ring and only its degradation is capable of releasing DNA entrapped by cohesin, both in *in vivo* and *in vitro* experiments. Moreover the cohesin complex dissociates from linearized minichromosomes (Gruber, 2003; Haering, 2002; Ivanov, 2005; Uhlmann, 1999). The alternative proposed model is the "handcuff model", in which two cohesin molecules, each surrounding a single sister chromatid, interact (Huang, 2005; N. Zhang, 2008).

### 1.6.3. Cohesin removal

Dissolution of sister chromatid cohesion requires careful timing in order to avoid mitotic arrest due to checkpoint activation, or incomplete chromatid separation and consequent aneuploidy.

In budding yeast cohesin removal takes place at anaphase, when the protease separase (Esp1) cleaves Scc1 on two specific residues (R268 and R269), thus opening the cohesin ring and releasing the sister chromatids that are now free to move following the pulling forces of the microtubules (Uhlmann, 1999). To avoid precocious cleavage by separase, its activity is inhibited by the regulatory protein securin, which is degraded through APC/C dependent ubiquitination, when the spindle checkpoint is inactivated. Scc1 is then re-synthetized in the following G1 phase.

## 1.7. COHESIN CYCLE IN METAZOAN

### 1.7.1. Cohesin loading

Metazoan cohesin loading shares with budding yeast the necessity of a loader (NIPBL-MAU2), and the requirement for ATP hydrolysis. Moreover in the same way as in *S. cerevisiae*, two different modes of interaction with DNA, before and after S phase, that differ in stability, can be observed for cohesin, by fluorescent recovery after photobleaching (FRAP) (Gerlich, 2006). However some differences can also be reported; a first discrepancy

concerns cell cycle timing of cohesin binding. In metazoan cohesin is present on chromatin from telophase to anaphase, while budding yeast cohesin loading occurs exclusively from late G1, after Scc1 is re-synthetized. Another difference is the interaction between NIPBL, cohesin and the mediator complex, required for the loading of cohesin at active promoters (Kagey, 2010). Lastly, in both metazoans and yeast, cohesin does not persist at the loading sites but is translocated to final sites of interaction, which however differ in character. In higher eukaryotes they are found at DNA sequences containing CCCTC-binding factor (CTCF) motifs, and not at regions of convergent transcription as in yeast (Parelho, 2008; Rubio, 2008; Stedman, 2008; Wendt, 2008).

### 1.7.2. Cohesion establishment

As mentioned before, after S phase, cohesin can be seen interacting more stably with DNA. Similar to budding yeast, acetylation of SMC3 is required to counteract the WAPL-PDS5 activity (J. Zhang, 2008). In metazoans two different orthologs of Eco1 exist, ESCO1 and ESCO2, both capable of acetylating SMC3, on the conserved residues K105/K106 and promoting sister chromatid cohesion (Hou, 2005; J. Zhang, 2008). The additional factor sororin, present in vertebrates, interacts with acetylated cohesin to further protect the complex from the action of WAPL (Nishiyama, 2010; Rankin, 2005; Schmitz, 2007).

### 1.7.3. Cohesin removal

In higher eukaryotes cohesin removal takes place in two separate steps. The majority of cohesin is removed from chromosome arms during prophase. Moreover in contrast to budding yeast also the cohesin associated proteins (WAPL PDS5, sororin, NIPBL/MAU2) are removed from chromosome arms. The prophase removal of cohesin is independent of kleisin cleavage, but is due to WAPL activity, and regulated by phosphorylation of SA2 by PLK1 and Aurora B in what is called the “prophase pathway”. Only centromeric cohesin remains intact till anaphase, when RAD21 is finally cleaved by separase (Giménez-Abián, 2004; Lénárt, 2007; Sumara, 2002; Waizenegger, 2000).

## 1.8. COHESIN & DNA DAMAGE REPAIR

Surprisingly the DNA repair function of cohesin was discovered prior to its role in sister chromatid cohesion. First, in *S. pombe*, Scc1 mutations were linked to UV and IR sensitivity. Later the role of cohesin in DNA damage repair was also discovered in *S. cerevisiae*, chicken and human (Atienza, 2005; Birkenbihl, 1992; Sonoda, 2001).

### 1.8.1. Budding yeast DNA damage response

In budding yeast, repair by HR requires the cohesin complex and the auxiliary factors for loading (Scc2) and establishment (PdS5, Eco1), pointing at a role for cohesion in DNA repair (Sjögren, 2001).

In an unchallenged cell cycle, after DNA replication, Eco1 is degraded due to a complex phosphorylation cascade. The proposed model is that Eco1 is first modified in early S phase by Cdk1 that primes, in late S phase, Cdc7-Dbf4 dependent phosphorylation which in turn determines Mck1 phosphorylation and consequent degradation (Lyons, 2011, 2013). For this reason the cohesin molecules, even though they can still be loaded onto DNA, cannot be made cohesive. In case of DNA damage, however, new cohesin is loaded and becomes cohesive, around the DSB and throughout the genome (Ström, 2004, 2007; Ünal, 2004, 2007). In order to achieve this damage-induced cohesion (DI-cohesion), Eco1 is stabilized due to Cdc7-Dbf4 inhibition (Lyons, 2013). Moreover Scc1 phosphorylation on serine 83 by Chk1, activated in turn by the Mec1 kinase, was suggested to drive acetylation by Eco1, necessary for G2 specific cohesion establishment (Heidinger-Pauli, 2008). DI-cohesion shows some differences compared to canonical cohesion establishment. First of all it does not require DNA replication since it is independent of Rad52 (Ström, 2007; Ünal, 2007). Moreover in a DNA damage situation Eco1 acetylates different residues than the canonical Smc3 sites (K112, 113), however it is still counteracted by Wpl1 activity (Heidinger-Pauli, 2008, 2009).

Binding of cohesin around a DSB covers approximately100 kb, except for a region of 5 kb in direct vicinity of the break, and depends on the MRX complex, Tel1, Mec1 and H2A phosphorylation (Ström, 2004; Ünal, 2004). The role of cohesin in DNA damage repair is still not clear but the most accredited hypothesis is that the complex keeps the DSB in close proximity to the undamaged DNA substrate in order to enable repair. Even though this concept is surely appealing for its intuitiveness it still needs to be proven.

### 1.8.2. Metazoan cohesin and DNA damage

Cohesin is recruited to DNA damage also in human cells, specifically in S/G2 phase, as shown in both immunofluorescence experiments with laser damage (J. S. Kim, 2002) and in ChIP experiments with I-Sce induced DSB (Potts, 2006). Moreover in human cells, in the same way as in budding yeast, cohesin likely requires the MRN complex as demonstrated by

experiments carried out on cell line lacking functional MRE11 and further strengthen by the finding of a direct interaction between the two complexes (J. S. Kim, 2002).

There are still no clear evidence that DI-cohesion is present also in metazoans but some data indicates that it might indeed be a conserved process. As a start, sororin which is required for cohesion formation, has been reported to be needed for DNA damage repair in G2 cells (Schmitz, 2007). Moreover, ChIP-seq mapping shows that, upon DNA damage induction, cohesin binding is reinforced at pre-existing binding sites, and ESCO1 dependent acetylation increases in quantitative mass spectrometry analysis (B. J. Kim, 2010).

Human cohesin has been also implicated in DNA damage checkpoint activation. The intra-S-phase checkpoint activation depends on ATM and ATR that directly phosphorylate SMC1 and SMC3 (Garg, 2004; Kitagawa, 2004; Luo, 2008; Yazdi, 2002). A role of cohesin in the G2/M checkpoint was confirmed by the fact that depletion of RAD21, affects foci formation of 53BP1 (Watrin, 2009).

NIPBL is as cohesin recruited to DNA damage, but this is a much less studied process. (discussed further below). Previous work have however shown that NIPBL recruitment to DNA damage depends on MCD1, RNF168 and HP1γ (Kong, 2014; Oka, 2011).

## 1.9. COHESIN AND MEIOSIS

The meiotic prophase I poses a big challenge for cells, since homologs need to undergo proper pairing and the formation of chiasmata and resolution of DSBs require perfect steric and topological control.

The cohesin complex is an absolute necessity for proper meiosis progression. In both yeasts and metazoan, meiosis specific cohesin subunits are different than in somatic cells. Budding yeast Scc1 is substituted by Rec8 (Klein, 1999), while mammalian SMC1α, RAD21 and SA1/2 are complemented by SMC1β, REC8, the newly discovered RAD21L and SA3 respectively. All these additional cohesin isoforms have specific binding patterns and non-redundant functions compared to their mitotic paralogs (Hopkins, 2014; Lee, 2011; Revenkova, 2001; Xu, 2005).

Observations from mouse meiosis showed Nipbl chromosomal association from zygotene until late pachytene, the same stages during which Rad21 containing cohesin complexes are

binding DNA. These evidences point to the possibility that meiotic cohesin loading is independent of replication.

The cohesin complex, aside from its canonical role of keeping the sister chromatids together, also supports the formation of the axial elements of the synaptonemal complex. Meiotic cohesin is released in two steps: first during anaphase I, separase cleaves cohesin from chromosome arms, while shugoshin protects centromeric cohesion until meiosis II, when cohesin is finally removed from sister chromatids, allowing their segregation (Rankin, 2015).

## 1.10. COHESION AND BEYOND

Several studies have shown that cohesin and its loader play a role in gene regulation, in addition to being essential for correct chromosomes segregation and DNA repair.

The first evidence for the involvement of the cohesin loader in transcription came from *D. Melanogaster*, where Nipped-B was found to promote long-range interactions between enhancers and promoters of the homeobox gene family (Rollins, 1999, 2004).

Other proofs came over the years from different models, in *C. Elegans* and *X. Laevis* Mau2, the ortholog of Scc4, is required for axon formation (Seitan, 2006). Similar results were obtained in *D. Melanogaster* where Nipped-B was linked to neuronal development (Pauli, 2008). However the most important evidence comes from humans where NIPBL was found frequently mutated in a developmental disorder called Cornelia de Lange syndrome (CdLS). These patients show multisystem malformations: short stature, intellectual disability, gastroesophageal dysfunction, growth defects of the upper limbs and distinctive facial features (Krantz, 2004; Tonkin, 2004).

Cell lines derived from CdLS patients and a mouse model of the syndrome, show defects in transcription but not in cell division, since cohesion is properly established (Castronovo, 2009; Revenkova, 2009). This is quite interesting, considering that also in budding yeast, the amount of available cohesin can be reduced to nearly 10% of wild type level before cell division defects become apparent (Heidinger-Pauli, 2010). This leads to the intriguing concept that the canonical role of cohesin in keeping sister chromatids together requires few active complexes, and the rest might be needed for other cellular functions.

Some evidence of a role for Scc2 in gene regulation can be found also in budding yeast. It has been reported that Scc2 directly affects Rec8 expression during meiosis (Lin, 2011).

Moreover, lack of Scc2 influences both general genome-wide and DNA damage response specific transcription (Lindgren, 2014). A possible model for gene regulation via Scc2 in yeast can be searched in the relationship between Scc2 and chromatin remodeling, since the RSC complex is needed for chromatin recruitment of the loader, and inhibition of Scc2 results in a similar transcription profile as RSC inhibition (Lopez-Serra, 2014).

Mechanistic insight on a role for Cohesin in gene regulation was identified in metazoans where it was reported to co-localize with CTCF, whose presence is also necessary for cohesin positioning. Cohesin on the other hand is required for proper CTCF function as an insulator affecting the expression of numerous genes (Parelho, 2008; Rubio, 2008; Stedman, 2008; Wendt, 2008).

It is still not clear how cohesin and its loader regulate gene expression, one possible explanation is that cohesin, with its ability to encircle DNA molecules, is capable of doing this also at an intramolecular level, together with CTCF, bringing two DNA sequences in close proximity, determining the formation of a loop (Sanborn, 2015) (Figure 4).

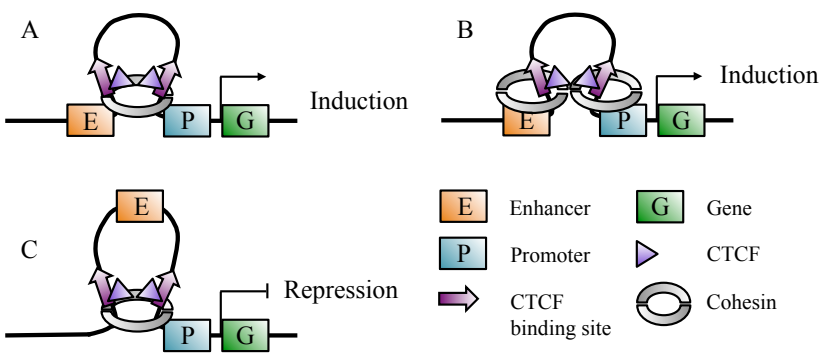


Figure 4: A Model on how cohesin can affect gene expression together with CTCF. Converging CTCF binding sequences determine the formation of a intramolecular loop stabilized by a single cohesin molecule encircling the loop (A). An alternative version of the model in which two cohesin molecules interact together to stabilize the intramolecular loop (B). Formation of a loop that impairs the interaction between the enhancer and the promoter (C).

# 2 METHODOLOGY

## 2.1 MODEL ORGANISMS

### 2.1.1 *Saccharomyces cerevisiae*

**Paper I** and **III** are based on invesigations in *S. cerevisiae*. Although there are some differences between higher eukaryotes and S. *cerevisiae*, it has the advantage of a short cell cycle (90 minutes under optimal conditions), easy growth conditions and genetic manipulation procedures that allow elaborate experiments with complex genetic backgrounds (for example long cell cycle arrest and multiple mutations or deletions). Compared to other eukaryotes *S. cerevisiae* has a compact genome comprising 6000 genes, with an average length of 1450 bp, divided on 16 rather short chromosomes (Dujon, 1996).

### 2.1.2 *Mus musculus*

In **Paper IV**, *M. musculus*, the most used vertebrate in biomedical studies is utilized. Thanks to a well known genome, a life span of 1-2 years, early sexual maturity, an average of 19 days of pregnancy and a litter size of 6 to 12 pups, mice are a practical mammalian model, relatively easy to modify, to breed and to grow. Especially for studies of meiosis *M. musculus* is the favored model organism. Gametogenesis (gamete formation) takes place in seminiferous tubules of the testes (spermatogenesis), for males, and in the ovaries (oogenesis) for females. While spermatogenesis is a continuous process where new gametes are produced every day, females have only a fixed number of primary oocytes generated during embryogenesis, These have the potential to become mature gametes, but are initially arrested in their development at the end of prophase I. During each menstrual cycle, due to a hormonal surge, they continue meiosis and become mature ova (Hess, 2008; Pepling, 2006). The female meiosis is completely finalized only at the moment of fertilization.

### 2.1.3 Human cell culture

Since research on humans for ethical reasons has multiple obvious restrictions, cell lines offer a good compromise to study human cellular processes *in vivo*.

The variety of available cell lines from different tissues, the possibility to administer drugs, like inhibitors for specific enzymes, and to knock down expression of genes of interest by siRNA, make cell cultures a powerful research tool.

In **Paper II** the commercial cell line HEK293 Flp-In™ T-REx™ (Thermo Fisher) was used. In this system cells contain the Flp recombination target (FRT) at a specific locus in the genome, which allows integration of a gene of interest, thanks to the Flp recombinase activity, consistently at the same position in the genome. We integrated various truncations and mutations of *NIPBL*, previously cloned in an expression vector, creating multiple stable cell lines. In this way experimental variation due to transfection and integration differences could be minimized.

## 2.2 IMMUNOFLUORESCENCE OF TESTICULAR AND OVARIAN NUCLEAR SPREADS

With nuclear spreads nuclei isolated from meiotic cells are distributed on a slide for visualization of chromosomes. We used an approach consisting of a drying-down technique that allows recovery of a sufficient number of nuclei and good preservation of chromatin structures (Peters, 1997). Mouse spermatocytes were obtained from young males, while prophase oocytes were obtained from mouse embryos (E16.5–E19.5). Testes and ovaries were dissected and then torn to pieces in order to make single cell suspensions. Nuclei extracted after incubation in a hypotonic buffer, were then fixed by spreading a drop of suspension on slides previously dipped in paraformaldehyde.

Immunofluorescence is a technique where antibodies are used to recognize a specific protein. The fluorescent signal derives from a secondary antibody, raised against the species of the primary antibody, carrying a fluorophore, a molecule that can emit light of a determined wavelength when excited by photons coming from a specific portion of the light spectrum. Specificity of antibodies used is of utmost importance in immunofluorescence, since it is impossible to tell by microscopy if the antibody binds off-target proteins. For this reason all non-commercial antibodies were also tested for specificity by western blot. Since in **Paper IV** we wished to compare genotypes, in order to reduce variation, the samples to be compared were prepared the same day, stained with the same antibody preparation and observed using the same microscope conditions.

## 2.3 MICROARRAY ANALYSIS

Microarrays are powerful tools with multiple possible applications, one of which is to measure transcript levels, comparing two different conditions, as in **Paper III**, where

differences in gene expression were observed in the presence or absence of DNA damage, comparing wild type cells and cells without functional Scc2. Arrays can be different in terms of manufacturing method, number of samples that can be profiled (single or double channel) and length of the probes (cDNA or oligonucleotides). In **Paper III** the GeneChip yeast genome 2.0 array from Affymetrix was used, a single channel oligonucleotide array. A limitation of microarrays is their strong sensitivity that can lead to variability in the raw data. The best course of action when performing a microarray-based study would be to reduce the sources of variability (batch, experimental variation, operator) to a minimum, which is often for logistic reasons not possible. This is even more problematic when comparing multiple conditions, with the possibility of failed experiments or outliers. For this reason it is important to pre-process the data obtained from the array. Pre-processing includes different steps: background correction, quantile normalization and summarization. Background correction is carried out to remove the effect of aspecific binding while quantile normalization is the statistical process used to compare two distributions. Summarization is on the other hand, the process that gives the expression values of a single gene derived from the data collected from multiple probes (Wu, 2009).

## 2.4 CHROMATIN IMMUNOPRECIPITATION

In **Paper I**, to detect Scc2 binding at the site of a DSB, chromatin immunoprecipitation (ChIP) coupled to quantitative real-time PCR was used (Figure 5). ChIP is the immunoprecipitation of a specific protein (in our case a tagged version of Scc2) where the whole cell extract derives from the lysis of cross-linked cells. This allows the proteins to stay linked to the DNA sequence they bind *in vivo*. In order to get good resolution in qPCR, chromatin is kept at an optimal size range of 300 to 500 bp, thanks to the sonication of the lysate prior of the immunoprecipitation. After protein-DNA complexes are eluted from the beads of the IP, they are de-crosslinked and DNA is then further purified for removal of proteins and RNA (Katou, 2006).

Purified DNA can then be analyzed using different approaches: tiling microarrays (ChIP-on-chip), massive parallel sequencing (ChIP-seq) or ChIP coupled to quantitative PCR (ChIP-qPCR). Out of the three, ChIP-qPCR offers fully quantitative data with the downside that it is possible to look only at few loci at a time. ChIP-on-chip and ChIP-seq allow a genome-wide analysis of the chromosomal association of a specific protein in a single experiment. In our

case however ChIP-qPCR is the perfect tool to observe binding levels of a specific protein around the site of a specific DNA DSB.

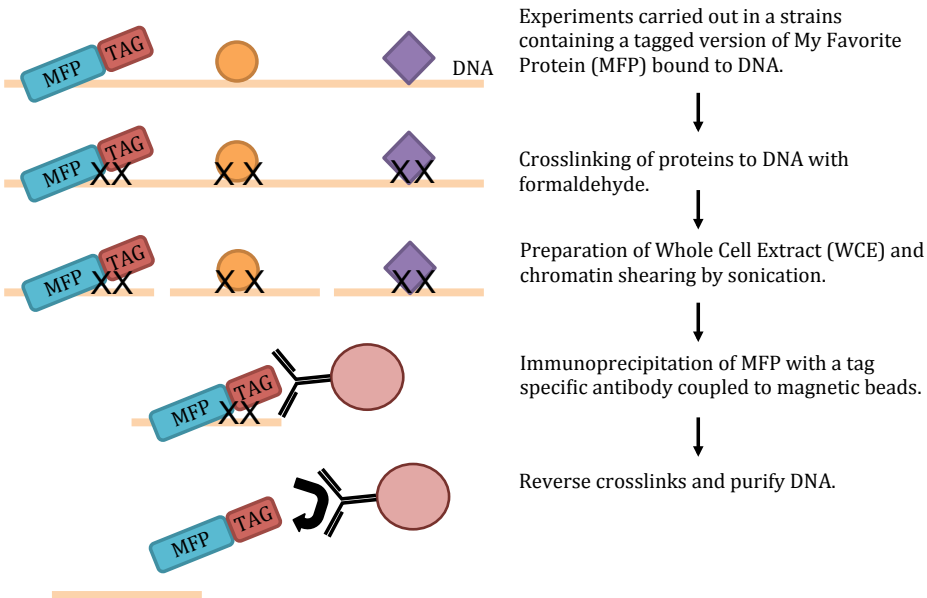


Figure 5: Scheme of the different steps of the ChIP assay.

## 2.5 DNA DAMAGE INDUCTION

In **Paper I**, **II** and **III** different approaches are used to induce DNA damage and observe the effects and the dynamics that follow this event.

In **Paper I** and **III** the homothallic switching endonuclease (HO) was used to induce DSBs in an artificially inserted recognition cut-site on chromosome VI of the yeast genome. The endonuclease was regulated by a strong inducible promoter (GAL10), replacing the endogenous HO promoter, in order to express the HO endonuclease by adding galactose to liquid media.

In **Paper II**, two different methods to produce DNA lesions were used. The first one is laser microirradiation, a method with a long history in the field of DNA damage effects in cells. In our particular case cells are pre-treated with either BrdU or TMP to sensitize them towards laser treatment. Different types of laser, with different sources and power output can be used

in order to induce different types of DNA damage. We used a laser output of 20 Hz, 364 nm that might generate interstrand cross-links in addition to DSBs, for this reason we used also a second method to create “clean” DSB: The FokI endonuclease.

The FokI reporter system is based on Lac operator repeats to which an inducible FokI endonuclease, fused to a Lac repressor and mCherry, is able to bind and induce DSBs. Expression of FokI was regulated in a similar way as the HO system, but in this case a destabilization domain and a modified estradiol receptor fused with the endonuclease in combination with addition of small molecules (Shield1 ligand and 4-OHT) was responsible for activation (Tang, 2013).

These three different approaches produce different effects, laser microirradiation for example induce DSB formation, but likely also other forms of DNA damage. On the other hand endonucleases produce clean DSBs, but while the HO endonuclease with a single cut-site induce a single DSB, the FokI endonuclease induces multiple breaks in the Lac operator region, which elicits a more robust response, possible to study with immunofluorescence microscopy.

# 3 RESULTS AND DISCUSSION

The four papers in this thesis are centered around the cohesin loading complex in different models and in different aspects of genome stability.

## 3.1 PAPER I

**Requirements for DNA Double Strand Break Accumulation of Scc2, Similarities and Differences with Cohesin**

Previous chapters have described a central role for cohesin in maintenance of genome stability, not only for proper chromosome separation but also during DNA damage. It is clear that it is not cohesin per se that is important, but cohesion establishment. It should however not be forgotten that both during an unchallenged cell cycle and under DNA damage conditions, cohesion requires proper cohesin loading through the heterodimer complex $NIPBL^{Scc2}/MAU2^{Scc4}$.

Previous work has reported Scc2 localization at DSB (Ström, 2007), the requirements for this are however still entirely unknown. The aim of this study was therefore to understand what is needed for Scc2 binding at DNA damage.

We first characterized Scc2 recruitment at a HO-induced DSB with a ChIP-based quantitative assay (ChIP-qPCR). Our data showed that Scc2 binding is enhanced up to 30 kb from the break site, with a strong enrichment around 5 kb, compared to unchallenged cells. We next addressed which of the factors reported to influence cohesin DSB binding, under similar DNA damage condition, that would also affect Scc2 DSB recruitment. We started our analysis with a *MRE11* deletion and not surprisingly, lack of this subunit of the MRX complex, one of the early factors in HR, strongly reduced the Scc2 accumulation at the DNA DSB.

We then tested the possibility that the effect observed in *mre11Δ* strain, is due to other factors dependent on the MRX complex. A *TEL1* deletion also affected Scc2 binding at the DSB negatively, and we thus speculated that this kinase, which is activated early upon DNA damage, might either directly phosphorylate Scc2/4 or affect Scc2 levels through H2A phosphorylation. However, a mass spectrometry analysis performed on the purified loading

complex did not show any phosphorylated S/TQ sites that might relate to Tel1. In addition, the only Scc4 residue (T597) specifically modified in response to DNA damage, had no growth defect in the presence of the genotoxic drug MMS when mutated.

Since the *hta1-S129A and hta2-S129A* mutations, preventing phosphorylation of H2A, showed similar results as the *tel1Δ,* it was possible to assume that the role of the Tel1 kinase is to create a scaffold of phosphorylated histones for recruitment of Scc2. However, it is not possible to completely rule out the need for a direct phosphorylation on either Scc2 or Scc4, and it would certainly be interesting to also test different approaches to address this possibility.

Since H2A phosphorylation is dependent also on Mec1, we next addressed the role of this second kinase, activated as part of the DNA damage response, and also necessary for cohesin binding at break sites (Ünal, 2004). However, unlike for cohesin, a *MEC1* deletion has no effect on Scc2 localization at DSB. Considering that Mec1 is recruited to ssDNA bound by RPA, and formed as a result of resection of DSB ends, we then tested the dependency of initial end-processing for Scc2 recruitment. Start of resection of an HO DSB is independent of Mre11 endonuclease activity, but depends on Sae2. In a strain with *SAE2* deletion background the Scc2 levels around the induced HO DSB are unchanged, thus resection has no effect on Scc2 recruitment.

This leads to some intriguing considerations, first of all it would be interesting to test if end-resection is required for cohesin binding, which could explain the different effects observed in *mec1Δ* strains between cohesin and its loader. In this respect it should be remembered that Mec1 is recruited to DSBs upon start of resection. Another open question is why Mec1 affects cohesin loading at DSBs, but not Scc2 localization. It is possible to speculate that Mec1 might be necessary to actively drive the cohesin loading process in case of DNA damage, either acting directly on cohesin or indirectly on the loader or even on something else yet to be defined. This could mean that Mec1 is required to drive a specific DNA damage dependent, postreplicative, cohesin loading, if not genome-wide at least around break sites.

In the last part of this paper we focused on elucidating, in more depth, the role of the MRX complex. Since resection was shown to be unnecessary for Scc2 binding and Scc2 levels were reduced in the absence of Xrs2, known to be required for Tel1 recruitment (Nakada, 2003), it is possible to assume that MRX is needed only to recruit other factors of the DNA damage response cascade. However, it should be noted that Xrs2 also partially affects the

MRX bridging function (Trujillo, 2003) and reduced Scc2 binding at break sites in a *rad50Δ* background seems to point in the direction of an active role for the MRX complex. It would be interesting to observe a direct recruitment of Scc2 by the MRX complex, similar to the physical interaction between cohesin and MRN that has been reported in human cells (J. S. Kim, 2002), and if that is the case, which subunit of the MRX complex is involved.

In conclusion, we would like to suggest that the MRX complex bridging activity and likely a Tel1 interaction are required for Scc2 binding at DNA breaks. Moreover it is possible to speculate on a model where Scc2 arrives early after damage induction, before resection starts, while cohesin is likely to be recruited to the DSB after resection is initiated, as can be inferred by the two different profiles of Scc2 and cohesin at DSBs. The first one is binding on the DNA sequence next to the break, similarly to Mre11, while cohesin is leaving a gap around the DSB similarly to the pattern of H2A phosphorylation.

## 3.2 PAPER II

**Independent Mechanisms Recruit the Cohesin Loader Protein NIPBL to Sites of DNA Damage**

In this study, similarly to **Paper I,** we aimed to understand the requirements for DNA damage recruitment of the cohesin loader. However here we investigated NIPBL, the human ortholog of Scc2, thus shifting from budding yeast to human cell lines. Previous work has described the recruitment of a transiently transfected, Halo tagged NIPBL at I-PpoI dependent DSBs in the region of 28S rDNA. In **Paper II** on the other hand we used confocal microscopy paired with laser microirradiation and the FokI endonuclease for DNA damage induction. Moreover a system comprised of HEK-293 cells stably transfected with inducible GFP tagged NIPBL, through a tetracycline repressor system, was generated.

We first showed in HEK-293 cells, that both NIPBL isoforms, A (316 kDa) and B (304 kDa), are recruited to laser damage and FokI induced breaks.

A MAU2 GFP tagged trans-gene was also recruited to laser damage, but did not form visible foci in the FokI system, likely due to the fact that MAU2 requires NIPBL to localize to the nucleus, confirmed by the fact that the ample majority of ectopic MAU2 remains cytoplasmic. Moreover, MAU2, at least in response to DNA damage, fails to act as chromatin adaptor of the loading complex, since NIPBL proteins carrying a missense mutation (glycine 15 to arginine) that disrupts the NIPBL-MAU2 interaction, still localized at H2AX foci, both at laser damage tracks and FokI induced breaks.

We next tested whether a NIPBL mutation, located inside the HP1 binding motif localized in the N-terminal part of NIPBL, had any affect on DNA damage localization of NIPBL, a PxVxL to PxAxA modification is known to abolish the interaction between NIPBL and HP1, and in doing so preventing NIPBL recruitment to I-PpoI induced DSB. We found that $NIPBL^{PxAxA}$ did not form foci in the FokI system, but that recruitment to laser damage still occurred, which led us to think of an additional, HP1 independent, recruitment mechanism for NIPBL. To further investigate and understand this dual mechanism multiple truncations of NIPBL were generated (Figure 6).

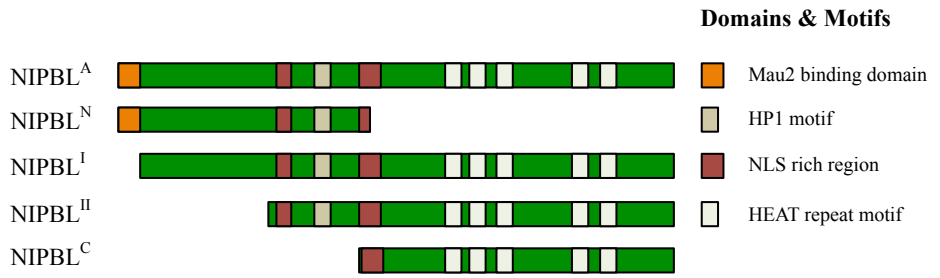


Figure 6: Scheme of the different NIPBL truncations used in **Paper II**. Different color boxes represent domains and motifs according to the legend.

While all truncations were recruited to laser damage, only NIPBL fragments containing the HP1 binding motif were recruited to FokI foci. This was also confirmed by depletion of HP1γ where the same NIPBL truncations did not localize at DNA damage.

In order to better understand the nature of these independent recruitment pathways, we evaluated the role of ATM and ATR, known to be required, in yeast, for cohesin loading at DNA damage. Using specific inhibitors for ATM (KU-60019) and ATR (AZD6738) we observed that single inhibition had no effect, while double inactivation of ATM and ATR showed reduced recruitment of $NIPBL^C$ but had no effect on $NIPBL^N$. It would be interesting to understand the role of ATM/ATR on the cohesin loader further, if they act directly on NIPBL or as part of a cascade involving additional factors? Certainly there are a few S/TQ sites in NIPBL found phosphorylated in mass spectrometry that could be interesting to mutate in order to test their effect in DNA damage recruitment (Stokes, 2007).

Following the same thread we wanted to investigate if the ubiquitin ligase pathway starting with RNF168, and reported to affect NIPBL recruitment to I-PpoI induced DNA breaks (Oka, 2011), had an effect on NIPBL recruitment in our systems. We showed that reduction of free ubiquitin, obtained by inhibiting the proteasome activity with MG132 caused reduced accumulation of $NIPBL^N$ and $NIPBL^C$ at laser damage lines, which is in line with a possible involvement of the ubiquitin ligases RNF8 and RNF168. Furthermore, cells depleted of RNF8 and RNF168 failed to accumulate both $NIPBL^N$ and $NIPBL^C$ at DNA damage. Various hypotheses can be formulated to explain the effect of RNF8/RNF168 depletion and proteasome inhibition on the recruitment of NIPBL. A first explanation could be a direct ubiquitination of NIPBL, which can be proven by ubiquitin pull-down coupled to NIPBL detection. A possible follow up would be to discover by MS the potentially modified

residues, understanding the effect of ubiquitination by mutating the cohesin loader on such residues and discover the E3 ligase responsible for the modification. A second possibility is the dependency of other factors in the RNF8/RNF168 cascade such as 53BP1 or RAP80, for DNA damage recruitment of NIPBL.

In conclusion this paper shows a dual mechanism for NIPBL recruitment, one dependent on HP1 and another dependent on ATM/ATR. Moreover we have described a link between ubiquitination and RNF8/RNF168, with NIPBL binding at DNA damage. There are of course many open questions regarding the dual mechanism for NIPBL damage recruitment. Does it depend on different types of damage? Or is it a redundant mechanism to guarantee that NIPBL is recruited at DNA damage.

### 3.3 PAPER III

**Inactivation of the Budding Yeast Cohesin Loader Scc2 alters Gene Expression both Globally and in Response to a Single DNA Double Strand Break.**

In **Paper III** we aimed to understand if Scc2 influenced gene expression in budding yeast, in the presence and absence of DNA damage.

To achieve that we made use of a microarray, testing over 5800 open reading frames (ORFs) comparing wild type cells with a strain containing a temperature sensitive allele for Scc2, in the presence or absence of DNA damage, in form of a single DSB on chromosome VI (Figure 7 experiment 1).

The transcriptional profiles showed that when comparing Scc2-deficient cells to wild type, both in the presence or absence of DNA damage, 754 and 567 probe sets respectively, were significantly affected. However 399 probe sets that showed differential expression were in common with or without DSB, which left 168 probe sets uniquely affected in response to DNA damage and 355 with lack of DNA damage, when comparing wild type and scc2 deficient cells.

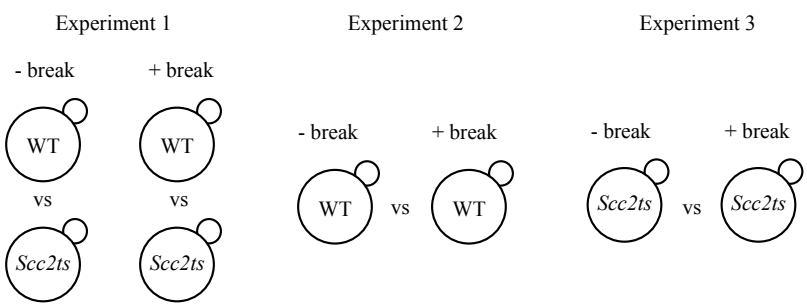


Figure 7: Three different microarray analysis with the experimental conditions used.

Genes with FDR≤0.05 were considered to significantly deviate from the expected genome frequency. Affected genes were then categorized based on biological process using the Saccharomyces Genome Data base Gene Ontology (SGD GO) slim mapping. Our findings showed that even though a majority of genes with altered expression, between *scc2-4* and wild type cells, were not involved in break induction, three pieces of evidence pointed to Scc2 also affecting the transcriptional response caused by DNA damage. First, the number of

affected genes was higher in wild type than in the *scc2* mutant in the presence of DNA damage, thus cells lacking functional Scc2 are likely incapable of proper DNA damage dependent transcriptional changes. Moreover when looking at the profiles and comparing presence or absence of DNA break, from cells lacking functional Scc2, the down-regulated genes belonged to categories such as; enhanced processes for "DNA damage", "DNA repair" and "DNA recombination". Wild type cells on the contrary, had several up-regulated genes of the DNA damage response but only in the presence of the DSB. In order to better understand the effect of Scc2 in the DNA damage transcriptional response it was necessary to study lack of Scc2 in isolation, comparing presence or absence of HO induction, without including wild type cells in the same experiment, since it was evident from the initial experiment that the transcriptional profiles from wild type and Scc2 deficient cells were so different that the alteration in gene transcription caused by a single DSB in Scc2 deficient cells were then masked.

In order to do that it was necessary to make sure, that a single break on chromosome VI was enough to cause a typical transcriptional change in DNA repair related genes, similarly to what has been previously reported for other DNA damage inducing agents. We thus initially tested the effect of the single DSB on wild type cells (Figure 7 experiments 2). Our findings showed that both the number (113) and type of affected genes were in accordance with existing data.

Since our system reflected a standard DNA damage response situation, we analyzed the effect of DNA damage on gene expression in *scc2-4* cells and found 976 altered genes (Figure 7 experiment 3). Many of the traditionally induced genes of the DNA damage response were upregulated, similarly to what was observed in wild type cells of the previous experiment (2). However a clear difference for genes of the cohesin network could be detected between wild type and *Scc2-4* cells.

We then further analyzed the two data sets (experiments 2 and 3) using SGD GO slim mapping. As expected in wild type cells the most enhanced processes were "cellular response to DNA damage stimulus" and "DNA repair". For the Scc2 mutant cells however, even though some of the genes classically induced upon DNA damage were upregulated, none of the processes enhanced in wild type cells could be observed. Instead other processes were affected, like "response to chemical stimuli", "oxidative stress" and "starvation". Moreover ribosome production was impaired in the *scc2-4* cells. Very interestingly, a similar effect was observed in a zebrafish model for CdLS and in Eco1 mutants, pointing out that the cohesin

network can be responsible for the ribosomal processes by affecting rRNA production (Lu, 2014; B. Xu, 2015).

In conclusion, it appears that Scc2 seems to have a role in maintaining gene regulation across the genome, in line with other results both for metazoans and also for yeast (Lopez-Serra, 2014). It is not yet clear however if in our case this function is independent of cohesin and might relate to the fact that Scc2 binds active promoters, or is a consequence of altered cohesin binding.

Our ChIP-seq maps, where we compared Scc1 chromatin association genome wide in unchallenged cells versus after induction of DNA damage, did not display any difference. It should be noted however that the binding pattern reflects binding of Scc1 in pre-replication loaded cohesin as well as complexes loaded in response to DNA damage. It would have been more relevant to look at G2 specific break induced cohesin loading. Even though genome wide cohesin binding did not change upon Scc2 inactivation, cohesin surrounding the break site was affected. As previously reported genes next to the break had reduced expression in wild type cells, after break induction, but only three out of six genes close to the break as tested by qRT-PCR, were repressed upon DNA damage in *scc2-4* cells. This result might indicate a possible effect of cohesin and its loader in silencing of gene expression around the break.

## 3.4 PAPER IV

**Localisation of the SMC loading complex Nipbl/Mau2 during Mammalian Meiotic Prophase I**

SMC complexes are master regulators of chromosome stability, not only in mitosis but also during meiosis. Still very little is known about the role of the cohesin loader NIPBL/MAU2 in meiosis.

In **Paper IV** we investigated the NIPBL/MAU2 chromosomal localization during both male and female mouse meiosis. Moreover, by using a mouse model for CdLS we aimed at addressing the effect of lacking one copy of Nipbl.

Staining of Nipbl and Mau2 in spermatocytes from testicular spreads showed an increasing intensity during prophase I, and relocation from chromosomal axes in leptotene to chromocentres in mid-pachytene. In oocytes derived from female embryos between E16.5 and E19.5 displayed a similar initial Nipbl and Mau2 distribution, with the clear difference that the complex was retained on chromosome axes during pachytene and showed a diffuse staining in the nucleus, unseen in male meiosis. This difference is quite interesting and it is tempting to explain it with a mechanism that requires Nipbl/Mau2 to maintain cohesion during dictyate, a condition where oocytes can stay for months.

The loading complex showed no co-localization with the meiosis specific cohesin subunit Smc1β, moreover it was interesting to note that cohesin, but not NIPBL, remained on chromosome axes after pachytene.

In spermatocytes derived from Nipbl$^{+/-}$ mice, a reduction of Nipbl levels in both western blot and staining intensity was observed. However Nipbl binding was only partly affected and cohesin was loaded as in wild type. We could conclude that lack of one functional copy of Nipbl did not affect cohesin loading as in somatic cells and certainly had no effect on maintaining cohesin binding at chromosomal stages later than zygotene. A similar effect was also true for other SMC complexes, neither condensin nor Smc5/6 staining changed in mutant mice. This lack of difference can possibly be explained for condensin and Smc5/6, with the fact that Nipbl might have no impact on loading of other SMC complexes than cohesin in mouse meiosis. Certainly more surprising is the lack of effect on cohesin, however this can be due to the fact that cohesin loading, at least for chromosome segregation, is unaffected by Nipbl gene dosage. Another possible reason is that variations are too small to be detected with immunofluorescence.

In Sycp1$^{-/-}$ and Sycp3$^{-/-}$ male and female germ cells, chromosomal axes are more disorganized and Nipbl/Mau2 had weaker intensity on the axes and were more diffuse in the nuclei. However Nipbl binding could still be observed even in the absence of SC components, possibly indicating that NIPBL binds prior to the SC formation.

Given the role of cohesin and its loader in DSB repair we checked the localization of γH2AX and Rad51 in relation to Nipbl. It appeared that the majority of the loader bound independently of these DNA damage and recombination markers. This held true in both wild type and Nipbl$^{+/-}$ mutant germ cells as well as in MEFs with irradiation induced γH2AX foci. However an interesting difference was observed between wild type and Nipbl$^{+/-}$ spermatocytes. From late zygotene γH2AX staining normally starts to disappear from the nucleus and is visible only at the sex body. In Nipbl$^{+/-}$ spermatocytes however, γH2AX nuclear staining was still strong even at mid-pachytene. This difference, together with the fact that reduction of Smc1β results in irregular γH2AX foci at late prophase, can mean that unlike canonical cohesin loading, localization of cohesin at meiotic DSBs is affected by Nipbl dosage. However DNA repair defects were not on the same level as in mitotic cells with altered γH2AX organization (Vrouwe, 2007). This could point to the possibility that meiotic cells can somehow protect themselves from the reduced Nipbl levels. An additional indication is that lethality in Nipbl$^{+/-}$ mice, as previously reported, is a post-natal event, taking place between conception and weaning (Kawauchi, 2009) while it seems that the majority of adult male mice are fertile. Lack of offspring for certain animals might depend on physical or behavioral alteration.

# 4 FUTURE PERSPECTIVE

## 4.1 HOW DOES THE LOADER WORK

Even though the importance of NIPBL$^{Scc2}$ is apparent, its mechanism of action is, for most parts, still unclear. Over the years significant details were uncovered, such as the possible entry gate of cohesin or the effect of ATP hydrolysis in modifying the cohesin ring conformation to allow DNA entry. The most important question however still remains: how cohesin is loaded onto DNA.

Previous works have shown the necessity for ATP hydrolysis in cohesin loading (Hu, 2011), moreover it appears that the cohesin loader stimulates the reaction carried out by the cohesin HEAD domain (Murayama, 2014). A possible model for loading is that ATP hydrolysis induces conformational changes in the coiled-coiled domain of the SMC proteins ultimately leading to the opening of the HINGE (Gruber, 2006). However, recent work has proposed a different model of loading in which entry and exit gates coincide in the region of contact between the kleisin subunit and Smc3. A conformational change induced by the loader will turn the cohesin ring "inside out" in order for the residues responsible of sensing DNA to contact the nucleic acid. This is possible thanks to the interaction of Scc2 with the HINGE of Smc3 (Murayama, 2015). Not unlikely more clues are hidden in the Scc2 structure, yet to be resolved.

Recent studies have managed to obtain Pds5 crystals (B.-G. Lee, 2016; Muir, 2016; Ouyang, 2016), the cohesin associated protein that shares with Scc2 a repetition of HEAT domains. This similarity might be of help in resolving the structure of Scc2 and/or NIPBL which can give useful information regarding the region of contacts between cohesin and its loader, since the core ring structure of cohesin is well characterized. As a matter of fact the domains in the cohesin subunits that bind the loader are known in *S. pombe*, but not vice versa. Mutation analysis of residues with functional importance can tell us more of the conformational changes induced by the loader and the effect on ATP hydrolysis.

Understanding the mechanism of Scc2 in *S. cerevisiae* can obviously be of great importance also for NIPBL studies. As mentioned before, the human cohesin loader has likely additional functions, or forms of regulation, that mirror the necessity of a genome with a higher degree of complexity. By understanding the conserved loading process, and the regions of the

protein that are involved in it, it will be possible to dissect the additional roles of NIPBL, potentially the most interesting will be those important for gene regulation.

One crucial region of the NIPBL$^{Scc2}$ protein is the C-terminal that has been suggested to be the cohesin-binding region (Kogut, 2009). In our case tagging of NIPBL at the C-terminal greatly destabilized the protein, which seems to strengthen this possibility. Thus either the C-terminal end is functionally important, or a highly ordered structure is destroyed by addition of an affinity tag. A similar effect is however not seen for Scc2, despite that it is the C-terminal part that is more conserved. It would certainly be interesting to better understand this difference and define precisely what region of the protein that is required for proper cohesin loading.

Another interesting fact is that cohesion seems independent of the available concentration of NIPBL in the cell (Castronovo, 2009). At the same time NIPBL haploinsufficiency causes multiple and serious developmental defects (Krantz, 2004; Tonkin, 2004). Even more surprisingly also duplication of NIPBL leads to disease (Novara, 2013), even though with a different phenotype than CdLS. This seems to strengthen the possibility that NIPBL is required in precise amount, and possibly not acting simply as the cohesin loader.

## 4.2 THE MYSTERY PROTEIN: MAU2$^{SCC4}$

Another intriguing issue concerns MAU2$^{Scc4}$; to this date it is still not clear what the true function of this second subunit of the cohesin loader is.

MAU2$^{Scc4}$ is an essential protein, however it is quite interesting to notice that no CdLS patients with MAU2 mutations have been found so far. Is this because MAU2 has no relevant function in the complex, or during development? Or is it possibly so that MAU2 mutations completely impair the activity of the cohesin loader? determining prenatal death. Or do we need to consider the possibility that MAU2 mutations can lead to a disease with a completely different phenotype than CdLS?

The only CdLS mutation that can somehow relate to this situation is the NIPBL G15R mutation that disrupts the interaction between NIPBL and MAU2 (Braunholz, 2012). In **Paper II** we have shown the effect of this mutation in NIPBL recruitment in case of DNA damage, however it should be remembered that G15R causes CdLS with a mild phenotype without heart and limbs defects, and it is possible that some residual interaction, not detectable with yeast-two-hybrid or western blot is enough to guarantee the complex

function. A more in depth characterization of this mutation, or additional ones affecting the interaction between NIPBL and MAU2, would be very interesting to carry out in order to better elucidate the relationship between MAU2 and NIPBL.

It is quite intriguing that an *in vitro* study on *S. pombe* Scc4 showed that it does not participate in any of the reported functions of the loader, neither ATP hydrolysis, nor DNA binding (Murayama, 2014). Another report in budding yeast did however show that Scc4 is required for cohesin loading at centromeres, and it would be interesting to see if similar results can be obtained also in human. It is possible to speculate that the main function of $MAU2^{Scc4}$ is to maintain the structure of the loading complex, or help the folding of $NIPBL^{Scc2}$. To support this hypothesis it should be remembered that MAU2 has no nuclear localization signal and that the formation of the complex should take place immediately after protein synthesis. The recently published structure of Scc4 can be important to address all these questions. Even though the small degree of conservation between Scc4 and MAU2 might make these studies difficult to transpose from yeast to human.

More data collected from Scc4 mutants can give us information regarding its interaction with kinetochore subunits or the need for histone modifications that can explain the cohesin loader recruitment at centromeres. More effort should also be put on finding possible suppressor mutants that can rescue an Scc4 deletion or temperature sensitive allele, which can point to other cellular pathways required for cohesin loading.

## 4.3 REMODELLING, TRANSCRIPTION AND COHESIN LOADING

A recent study showed a strong correlation between DNA binding of the cohesin loader and chromatin remodeling by the RSC complex (Lopez-Serra, 2014). This finding opens to various speculations. First of all is this mechanism conserved in metazoans? The fact that the Coffin-Siris Syndrome, caused by mutations in the gene encoding the human ortholog of RSC, has a similar phenotype as CdLS patients points in this direction. Finding CdLS patients lacking NIPBL, but carrying mutations in the chromatin remodeler genes, or Coffin-Siris Syndrome patients with NIPBL mutations would strengthen this concept.

This could also provide information on the relationship between the two complexes. Are they physically interacting, and if so, through which subunits and protein regions? Again structural information on Scc2 might help addressing these questions. Regardless, this link could strengthen the concept that cohesin is translocated to binding sites by transcription. However

this model still requires clarification. For example, some evidences point out the fact that cohesin sliding is strongly reduced by obstacles on DNA such as nucleosomes (Stigler, 2016). Moreover the RSC complex, more specifically Rsc2 and Rsc7, were found to be important for cohesin loading at an HO-induced break site (Oum, 2011). The connection between cohesin binding via the RSC complex, at DNA damage and during the unchallenged cell cycle seems clear and it is very possible that the described effect depends on Scc2. It would still be interesting to see which of the two existing RSC complexes that affect cohesin and its loader. The other intriguing possibility involves transcription and DNA damage. Loss of transcription can be observed in the vicinity of a DSB due to resection (Manfrini, 2015). A model for cohesin sliding via the transcription machinery is valid also during a DNA damage response? Thus, the similarity between the cohesin binding profile and H2A phosphorylation around the break is very intriguing. Does the gap in localization of the two in direct vicinity of the cut-site depend on resection, and the consequent lack of transcription? On the other hand why is Scc2 then located directly on the cut-site?

## 4.4 FINAL REMARKS

Soon the cohesin field will celebrate 20 years from the discovery of the complex that holds sister chromatids together. It is a relatively recent field of research however outstanding steps forward were made to understand one of the basic and yet so important mechanisms of life. Still there is more to discover: the role of cohesin in DNA damage, in gene regulation, and the correlation with replication and topology to mention some processes where the cohesin network has been suggested to play important roles.

This thesis describes different forms of action of the cohesin loader in different cellular processes. More data should be collected from mutants of conserved residues with a functional importance in cohesin, $NIPBL^{Scc2}$ and $MAU2^{Scc4}$ in order to dissect the various steps in the loading process or the different roles of each protein.

To select these mutants, large screenings utilizing protein arrays, studies of crystal structure, and deletion libraries should be carried out.

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# 6 REFERENCES

Arumugam, P., Gruber, S., Tanaka, K., Haering, C. H., Mechtler, K., & Nasmyth, K. (2003). ATP Hydrolysis Is Required for Cohesin's Association with Chromosomes. *Current Biology*, *13*(22), 1941–1953.

Atienza, J. M., Roth, R. B., Rosette, C., Smylie, K. J., Kammerer, S., Rehbock, J., Ekblom, J., & Denissenko, M. F. (2005). Suppression of RAD21 gene expression decreases cell growth and enhances cytotoxicity of etoposide and bleomycin in human breast cancer cells. *Molecular Cancer Therapeutics*, *4*(3), 361–8.

Aylon, Y., Liefshitz, B., & Kupiec, M. (2004). The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. *The EMBO Journal*, *23*(24), 4868–4875.

Beucher, A., Birraux, J., Tchouandong, L., Barton, O., Shibata, A., Conrad, S., Goodarzi, A. A., Krempler, A., Jeggo, P. A., & Löbrich, M. (2009). ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *The EMBO Journal*, *28*(21), 3413–27.

Birkenbihl, R. P., & Subramani, S. (1992). Cloning and characterization of rad21 an essential gene of Schizosaccharomyces pombe involved in DNA double-strand-break repair. *Nucleic Acids Research*, *20*(24), 6605–11.

Braunholz, D., Hullings, M., Gil-Rodríguez, M. C., Fincher, C. T., Mallozzi, M. B., Loy, E., Albrecht, M., Kaur, M., Limon, J., Rampuria, A., Clark, D., Kline, A., Dalski, A., Eckhold, J., Tzschach, A., Hennekam, R., Gillessen-Kaesbach, G., … Kaiser, F. J. (2012). Isolated NIBPL missense mutations that cause Cornelia de Lange syndrome alter MAU2 interaction. *European Journal of Human Genetics*, *20*(3), 271–276.

Buheitel, J., & Stemmann, O. (2013). Prophase pathway-dependent removal of cohesin from human chromosomes requires opening of the Smc3-Scc1 gate. *The EMBO Journal*, *32*(5), 666–76.

Castronovo, P., Gervasini, C., Cereda, A., Masciadri, M., Milani, D., Russo, S., Selicorni, A., & Larizza, L. (2009). Premature chromatid separation is not a useful diagnostic marker for Cornelia de Lange syndrome. *Chromosome Research*, *17*(6), 763–771.

Chan, K. L., Roig, M. B., Hu, B., Beckouët, F., Metson, J., & Nasmyth, K. (2012). Cohesin's DNA exit gate is distinct from its entrance gate and is regulated by acetylation. *Cell*, *150*(5), 961–974.

Chao, W. C. H., Murayama, Y., Muñoz, S., Costa, A., Uhlmann, F., & Singleton, M. R. (2015). Structural Studies Reveal the Functional Modularity of the Scc2-Scc4 Cohesin Loader. *Cell Reports*, *12*(5), 719–25.

Chen, F., Nastasi, A., Shen, Z., Brenneman, M., Crissman, H., & Chen, D. J. (1997). Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. *DNA Repair*, *384*(3), 205–211.

Ciosk, R., Shirayama, M., Shevchenko, A., Tanaka, T., Toth, A., Shevchenko, A., & Nasmyth, K. (2000). Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. *Molecular Cell*, *5*(2), 243–254.

Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005). The Saccharomyces cerevisiae Sae2 protein promotes resection and bridging of double strand break ends. *Journal of Biological Chemistry*, *280*(46), 38631–38638.

Clever, B., Interthal, H., Schmuckli-maurer, J., King, J., Sigrist, M., & Heyer, W. (1997). Recombinational repair in yeast : functional interactions between Rad51 and Rad54 proteins. *The EMBO Journal*, *16*(9), 2535–2544.

Davis, A. P., & Symington, L. S. (2001). The Yeast Recombinational Repair Protein Rad59 Interacts With Rad52 and Stimulates Single-Strand Annealing. *Genetics*, *159*(2), 515–525.

De Jager, M., Van Noort, J., Van Gent, D. C., Dekker, C., Kanaar, R., & Wyman, C. (2001). Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Molecular Cell*, *8*(5), 1129–1135.

Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D. H., Pepperkok, R., Ellenberg, J., Panier, S., Durocher, D., Bartek, J., Lukas, J., & Lukas, C. (2009). RNF168 Binds and Amplifies Ubiquitin Conjugates on Damaged Chromosomes to Allow Accumulation of Repair Proteins. *Cell*, *136*(3), 435–446.

Dujon, B. (1996). The yeast genome project: what did we learn? *Trends in Genetics : TIG*, *12*(7), 263–270.

Eckert, C. a., Gravdahl, D. J., & Megee, P. C. (2007). The enhancement of pericentromeric

cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. *Genes and Development*, *21*(3), 278–291.

Fernius, J., & Marston, A. L. (2009). Establishment of cohesion at the pericentromere by the Ctf19 kinetochore subcomplex and the replication fork-associated factor, Csm3. *PLoS Genetics*, *5*(9).

Fernius, J., Nerusheva, O. O., Galander, S., Alves, F. D. L., Rappsilber, J., & Marston, A. L. (2013). Cohesin-dependent association of Scc2/4 with the centromere initiates pericentromeric cohesion establishment. *Current Biology*, *23*(7), 599–606.

Fu, Y., Pastushok, L., & Xiao, W. (2008). DNA damage-induced gene expression in Saccharomyces cerevisiae. *FEMS Microbiology Reviews*, *32*(6), 908–926.

Garg, R., Callens, S., Lim, D.-S., Canman, C. E., Kastan, M. B., & Xu, B. (2004). Chromatin association of rad17 is required for an ataxia telangiectasia and rad-related kinase-mediated S-phase checkpoint in response to low-dose ultraviolet radiation. *Molecular Cancer Research : MCR*, *2*(6), 362–369.

Gasch, A. P., Huang, M., Metzner, S., Botstein, D., Elledge, S. J., & Brown, P. O. (2001). Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. *Molecular Biology of the Cell*, *12*(10), 2987–3003.

Gasch, A. P., & Werner-Washburne, M. (2002). The genomics of yeast responses to environmental stress and starvation. *Functional and Integrative Genomics*, *2*(4-5), 181–192.

Gerlich, D., Koch, B., Dupeux, F., Peters, J.-M., & Ellenberg, J. (2006). Live-cell imaging reveals a stable cohesin-chromatin interaction after but not before DNA replication. *Current Biology : CB*, *16*(15), 1571–8.

Gillespie, P. J., & and Hirano, T. (2004). Scc2 Couples Replication Licensing to Sister Chromatid Cohesion in in Xenopus Egg Extracts. *Current Biology*, *14*(17), 1598–1603.

Giménez-Abián, J. F., Sumara, I., Hirota, T., Hauf, S., Gerlich, D., de la Torre, C., Ellenberg, J., & Peters, J.-M. (2004). Regulation of Sister Chromatid Cohesion between Chromosome Arms. *Current Biology*, *14*(13), 1187–1193.

Glynn, E. F., Megee, P. C., Yu, H. G., Mistrot, C., Unal, E., Koshland, D. E., DeRisi, J. L., & Gerton, J. L. (2004). Genome-wide mapping of the cohesin complex in the yeast

Saccharomyces cerevisiae. *PLoS Biology*, *2*(9), E259.

Gottlieb, T. M., & Jackson, P. (1993). The DNA-Dependent Protein Kinase: Requirement for DNA Ends and Association with Ku Antigen. *Cell*, *72*(1), 131–142.

Gruber, S., Arumugam, P., Katou, Y., Kuglitsch, D., Helmhart, W., Shirahige, K., & Nasmyth, K. (2006). Evidence that Loading of Cohesin Onto Chromosomes Involves Opening of Its SMC Hinge. *Cell*, *127*(3), 523–537.

Gruber, S., Haering, C. H., & Nasmyth, K. (2003). Chromosomal cohesin forms a ring. *Cell*, *112*(6), 765–777.

Guacci, V., Koshland, D., & Strunnikov, A. (1997). A Direct Link between Sister Chromatid Cohesion and Chromosome Condensation Revealed through the Analysis of MCD1 in S . cerevisiae. *Cell*, *91*(1), 47–57.

Haering, C. H., Farcas, A.-M., Arumugam, P., Metson, J., & Nasmyth, K. (2008). The cohesin ring concatenates sister DNA molecules. *Nature*, *454*(7202), 297–301.

Haering, C. H., Löwe, J., Hochwagen, A., & Nasmyth, K. (2002). Molecular architecture of SMC proteins and the yeast cohesin complex. *Molecular Cell*, *9*(4), 773–788.

Handel, M. A., & Schimenti, J. C. (2010). Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. *Nature Reviews. Genetics*, *11*(2), 124–136.

Hartman, T., Stead, K., Koshland, D., & Guacci, V. (2000). Pds5p is an essential chromosomal protein required for both sister chromatid cohesion and condensation in Saccharomyces cerevisiae. *Journal of Cell Biology*, *151*(3), 613–626.

Heidinger-Pauli, J. M., Mert, O., Davenport, C., Guacci, V., & Koshland, D. (2010). Systematic Reduction of Cohesin Differentially Affects Chromosome Segregation, Condensation, and DNA Repair. *Current Biology*, *20*(10), 957–963.

Heidinger-Pauli, J. M., Ünal, E., Guacci, V., & Koshland, D. (2008). The Kleisin Subunit of Cohesin Dictates Damage-Induced Cohesion. *Molecular Cell*, *31*(1), 47–56.

Heidinger-Pauli, J. M., Ünal, E., & Koshland, D. (2009). Distinct Targets of the Eco1 Acetyltransferase Modulate Cohesion in S Phase and in Response to DNA Damage. *Molecular Cell*, *34*(3), 311–321.

Hess, R. A., & Franca, L. R. De. (2008). Spermatogenesis and Cycle of the Seminiferous

Epithelium. *Advances in Experimental Medicines and Biology*, *636*, 1–15.

Hinshaw, S. M., Makrantoni, V., Kerr, A., Ele, A., Marston, L., & Harrison, S. C. (2015). Structural evidence for Scc4-dependent localization of cohesin loading. *Elife*, *4*, e06057.

Hirano, M., & Hirano, T. (2002). Hinge-mediated dimerization of SMC protein is essential for its dynamic interaction with DNA. *The EMBO Journal*, *21*(21), 5733–5744.

Hirano, T., Kobayashi, R., & Hirano, M. (1997). Condensins, Chromosome Condensation Protein Complexes Containing XCAP-C, XCAP-E and a Xenopus Homolog of the Drosophila Barren Protein Tatsuya. *Cell*, *89*(4), 511–521.

Hirano, T., & Mitchison, T. J. (1994). A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell*, *79*(3), 449–458.

Hopkins, J., Hwang, G., Jacob, J., Sapp, N., Bedigian, R., Oka, K., Overbeek, P., Murray, S., & Jordan, P. W. (2014). Meiosis-Specific Cohesin Component, Stag3 Is Essential for Maintaining Centromere Chromatid Cohesion, and Required for DNA Repair and Synapsis between Homologous Chromosomes. *PLoS Genetics*, *10*(7), e1004413.

Hou, F., & Zou, H. (2005). Two Human Orthologues of Eco1/Ctf7 Acetyltransferases Are Both Required for Proper Sister-Chromatid Cohesion. *Molecular Biology of the Cell*, *16*(8), 3908–3918.

Hu, B., Itoh, T., Mishra, A., Katoh, Y., Chan, K. L., Upcher, W., Godlee, C., Roig, M. B., Shirahige, K., & Nasmyth, K. (2011). ATP hydrolysis is required for relocating cohesin from sites occupied by its Scc2/4 loading complex. *Current Biology*, *21*(1), 12–24.

Huang, C. E., Milutinovich, M., & Koshland, D. (2005). Rings, bracelet or snaps: fashionable alternatives for Smc complexes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *360*(1455), 537–542.

Huen, M. S. Y., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., & Chen, J. (2007). RNF8 Transduces the DNA-Damage Signal via Histone Ubiquitylation and Checkpoint Protein Assembly. *Cell*, *131*(5), 901–914.

Ivanov, D., & Nasmyth, K. (2005). A topological interaction between cohesin rings and a circular minichromosome. *Cell*, *122*(6), 849–860.

Ivanov, D., Schleiffer, A., Eisenhaber, F., Mechtler, K., Haering, C. H., & Nasmyth, K.

(2002). Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. *Current Biology*, *12*(02), 323–328.

Kagey, M. H., Newman, J. J., Bilodeau, S., Zhan, Y., Orlando, D. A., van Berkum, N. L., Ebmeier, C. C., Goossens, J., Rahl, P. B., Levine, S. S., Taatjes, D. J., Dekker, J., & Young, R. A. (2010). Mediator and cohesin connect gene expression and chromatin architecture. *Nature*, *467*(7314), 430–435.

Katou, Y., Kaneshiro, K., Aburatani, H., & Shirahige, K. (2006). Genomic Approach for the Understanding of Dynamic Aspect of Chromosome Behavior. *Methods in Enzymology*, *409*, 389–410.

Kawauchi, S., Calof, A. L., Santos, R., Lopez-Burks, M. E., Young, C. M., Hoang, M. P., Chua, A., Lao, T., Lechner, M. S., Daniel, J. A., Nussenzweig, A., Kitzes, L., Yokomori, K., Hallgrimsson, B., & Lander, A. D. (2009). Multiple organ system defects and transcriptional dysregulation in the Nipbl+/- mouse, a model of Cornelia de Lange syndrome. *PLoS Genetics*, *5*(9), e1000650.

Kegel, A., Betts-Lindroos, H., Kanno, T., Jeppsson, K., Ström, L., Katou, Y., Itoh, T., Shirahige, K., & Sjögren, C. (2011). Chromosome length influences replication-induced topological stress. *Nature*, *471*, 392–396.

Kim, B. J., Li, Y., Zhang, J., Xi, Y., Li, Y., Yang, T., Jung, S. Y., Pan, X., Chen, R., Li, W., Wang, Y., & Qin, J. (2010). Genome-wide reinforcement of cohesin binding at pre-existing cohesin sites in response to ionizing radiation in human cells. *Journal of Biological Chemistry*, *285*(30), 22784–22792.

Kim, J. S., Krasieva, T. B., LaMorte, V., Malcolm, A., Taylor, R., & Yokomori, K. (2002). Specific recruitment of human cohesin to laser-induced DNA damage. *Journal of Biological Chemistry*, *277*(47), 45149–45153.

Kitagawa, R., Bakkenist, C. J., McKinnon, P. J., & Kastan, M. B. (2004). Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes and Development*, *18*(12), 1423–1438.

Klein, F., Mahr, P., Galova, M., Buonomo, S. B. C., Michaelis, C., Nairz, K., & Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell*, *98*(1), 91–103.

Kogut, I., Wang, J., Guacci, V., Mistry, R. K., & Megee, P. C. (2009). The Scc2/Scc4 cohesin loader determines the distribution of cohesin on budding yeast chromosomes. *Genes and Development*, *23*, 2345–2357.

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., Panier, S., Mendez, M., Wildenhain, J., Thomson, T. M., Pelletier, L., Jackson, S. P., & Durocher, D. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science*, *318*(5856), 1637–1640.

Kong, X., Ball, A. R., Pham, H. X., Zeng, W., Chen, H.-Y., Schmiesing, J. A., Kim, J.-S., Berns, M., & Yokomori, K. (2014). Distinct functions of human cohesin-SA1 and cohesin-SA2 in double-strand break repair. *Molecular and Cellular Biology*, *34*(4), 685–98.

Krantz, I. D., McCallum, J., DeScipio, C., Kaur, M., Gillis, L. a, Yaeger, D., Jukofsky, L., Wasserman, N., Bottani, A., Morris, C. a, Nowaczyk, M. J. M., Toriello, H., Bamshad, M. J., Carey, J. C., Rappaport, E., Kawauchi, S., Lander, A. D., … Jackson, L. G. (2004). Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B. *Nature Genetics*, *36*(6), 631–635.

Kueng, S., Hegemann, B., Peters, B. H., Lipp, J. J., Schleiffer, A., Mechtler, K., & Peters, J. M. (2006). Wapl Controls the Dynamic Association of Cohesin with Chromatin. *Cell*, *127*(5), 955–967.

Lechner, M. S., Schultz, D. C., Negorev, D., Maul, G. G., & Rauscher, F. J. (2005). The mammalian heterochromatin protein 1 binds diverse nuclear proteins through a common motif that targets the chromoshadow domain. *Biochemical and Biophysical Research Communications*, *331*(4), 929–937.

Lee, B.-G., Roig, M. B., Jansma, M., Petela, N., Metson, J., Nasmyth, K., & Löwe, J. (2016). Crystal Structure of the Cohesin Gatekeeper Pds5 and in Complex with Kleisin Scc1. *Cell Reports*, *14*(9), 2108–2115.

Lee, J., & Hirano, T. (2011). RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. *Journal of Cell Biology*, *192*(2), 263–276.

Lehmann, A. R., Walicka, M., Griffiths, D. J. F., Murray, J. M., Watts, F. Z., Ready, S. M., & Carr, A. M. (1995). The rad18 Gene of Schizosaccharomyces pombe Defines a New Subgroup of the SMC Superfamily Involved in DNA Repair. *Molecular and Cellular Biology*, *15*(12), 7067–7080.

Lénárt, P., Petronczki, M., Steegmaier, M., Di Fiore, B., Lipp, J. J., Hoffmann, M., Rettig, W. J., Kraut, N., & Peters, J. M. (2007). The Small-Molecule Inhibitor BI 2536 Reveals Novel Insights into Mitotic Roles of Polo-like Kinase 1. *Current Biology*, *17*(4), 304–315.

Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G. P., Itoh, T., Watanabe, Y., Shirahige, K., & Uhlmann, F. (2004). Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature*, *430*(6999), 573–578.

Lengronne, A., McIntyre, J., Katou, Y., Kanoh, Y., Hopfner, K. P., Shirahige, K., & Uhlmann, F. (2006). Establishment of Sister Chromatid Cohesion at the S. cerevisiae Replication Fork. *Molecular Cell*, *23*(6), 787–799.

Lieber, M. R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annual Review of Biochemistry*, *79*, 181–211.

Lieber, M. R., Gu, J., Lu, H., Shimazaki, N., & Tsai, A. G. (2010). Nonhomologous DNA End Joining (NHEJ) and Chromosomal Translocations in Humans. *Sub-Cellular Biochemistry*, *50*, 279–296.

Lin, W., Jin, H., Liu, X., Hampton, K., & Yu, H.-G. (2011). Scc2 regulates gene expression by recruiting cohesin to the chromosome as a transcriptional activator during yeast meiosis. *Molecular Biology of the Cell*, *22*(12), 1985–1996.

Lindgren, E., Hägg, S., Giordano, F., Björkegren, J., & Ström, L. (2014). a single DNA double strand break Inactivation of the budding yeast cohesin loader Scc2 alters gene expression both globally and in response to a single DNA double strand break. *Cell Cycle*, *12*(23), 37–41.

Lisby, M., Barlow, J. H., Burgess, R. C., & Rothstein, R. (2004). Choreography of the DNA damage response: Spatiotemporal relationships among checkpoint and repair proteins. *Cell*, *118*(6), 699–713.

Lopez-Serra, L., Kelly, G., Patel, H., Stewart, A., & Uhlmann, F. (2014). The Scc2-Scc4 complex acts in sister chromatid cohesion and transcriptional regulation by maintaining nucleosome-free regions. *Nature Genetics*, *46*(10), 1147–51.

Lopez-Serra, L., Lengronne, A., Borges, V., Kelly, G., & Uhlmann, F. (2013). Budding yeast Wapl controls sister chromatid cohesion maintenance and chromosome condensation. *Current Biology*, *23*(1), 64–69.

Lu, S., Lee, K. K., Harris, B., Xiong, B., Bose, T., Saraf, A., Hattem, G., Florens, L., Seidel, C., & Gerton, J. L. (2014). The cohesin acetyltransferase Eco1 coordinates rDNA replication and transcription. *EMBO Reports*, *15*(5), 609–617.

Luo, H., Li, Y., Mu, J. J., Zhang, J., Tonaka, T., Hamamori, Y., Sung, Y. J., Wang, Y., & Qin, J. (2008). Regulation of intra-S phase checkpoint by ionizing radiation (IR)-dependent and IR-independent phosphorylation of SMC3. *Journal of Biological Chemistry*, *283*(28), 19176–19183.

Lyons, N. A., Fonslow, B. R., Diedrich, J. K., Iii, J. R. Y., & Morgan, D. O. (2013). Sequential primed kinases create a damage-responsive phosphodegron on Eco1. *Nature Structural & Molecular Biology*, *20*(2), 194–201.

Lyons, N. A., & Morgan, D. O. (2011). Cdk1-Dependent Destruction of Eco1 Prevents Cohesion Establishment after S Phase. *Molecular Cell*, *42*(3), 378–389.

Manfrini, N., Clerici, M., Wery, M., Colombo, C. V., Descrimes, M., Morillon, A., d'Adda di fagagna, F., & Longhese, M. P. (2015). Resection is responsible for loss of transcription around a double-strand break in Saccharomyces cerevisiae. *eLife*, *4*, 1–15.

Melby, T. E., Ciampaglio, C. N., Briscoe, G., & Erickson, H. P. (1998). The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: Long, antiparallel coiled coils, folded at a flexible hinge. *Journal of Cell Biology*, *142*(6), 1595–1604.

Michaelis, C., Ciosk, R., & Nasmyth, K. (1997). Cohesins : Chromosomal Proteins that Prevent Premature Separation of Sister Chromatids. *Cell*, *91*(1), 35–45.

Milne, G. T., Jin, S., Shannon, K. B., & Weaver, D. T. (1996). Mutations in two Ku homologs define a DNA end-joining repair pathway in Saccharomyces cerevisiae. *Molecular and Cellular Biology*, *16*(8), 4189–98.

Mimitou, E. P., & Symington, L. S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature*, *455*(7214), 770–4.

Moldovan, G. L., Pfander, B., & Jentsch, S. (2006). PCNA Controls Establishment of Sister Chromatid Cohesion during S Phase. *Molecular Cell*, *23*(5), 723–732.

Muir, K. W., Kschonsak, M., Li, Y., Metz, J., Haering, C. H., & Panne, D. (2016). Structure of the Pds5-Scc1 Complex and Implications for Cohesin Function. *Cell Reports*, *14*(9), 2116–

2126.

Murayama, Y., & Uhlmann, F. (2014). Biochemical reconstitution of topological DNA binding by the cohesin ring. *Nature*, *505*, 367–71.

Murayama, Y., & Uhlmann, F. (2015). DNA Entry into and Exit out of the Cohesin Ring by an Interlocking Gate Mechanism. *Cell*, *163*(7), 1628–1640.

Nakada, D., Matsumoto, K., & Sugimoto, K. (2003). ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes and Development*, *17*(16), 1957–1962.

Neuwald, A. F., & Hirano, T. (2000). HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. *Genome Research*, *10*(10), 1445–1452.

New, J. H., Sugiyama, T., Zaitseva, E., & Kowalczykowski, S. C. (1998). Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature*, *391*(22), 407–410.

Ng, T. M., Waples, W. G., Lavoie, B. D., & Biggins, S. (2009). Pericentromeric Sister Chromatid Cohesion Promotes Kinetochore Biorientation. *Molecular Biology of the Cell*, *20*(17), 3818–3827.

Nimonkar, A. V, Genschel, J., Kinoshita, E., Polaczek, P., Campbell, J. L., Wyman, C., Modrich, P., & Kowalczykowski, S. C. (2011). BLM, DNA2, RPA, MRN and EXO1, BLM, RPA, MRN constitute two DNA end resection machineries for human DNA break repair. *Genes & Development*, *25*(4), 350–362.

Nishiyama, T., Ladurner, R., Schmitz, J., Kreidl, E., Schleiffer, A., Bhaskara, V., Bando, M., Shirahige, K., Hyman, A. A., Mechtler, K., & Peters, J. M. (2010). Sororin mediates sister chromatid cohesion by antagonizing Wapl. *Cell*, *143*(5), 737–749.

Novara, F., Alfei, E., D'Arrigo, S., Pantaleoni, C., Beri, S., Achille, V., Sciacca, F. L., Giorda, R., Zuffardi, O., & Ciccone, R. (2013). 5p13 microduplication syndrome: A new case and better clinical definition of the syndrome. *European Journal of Medical Genetics*, *56*(1), 54–58.

Oka, Y., Suzuki, K., Yamauchi, M., Mitsutake, N., & Yamashita, S. (2011). Recruitment of the cohesin loading factor NIPBL to DNA double-strand breaks depends on MDC1, RNF168

and HP1ϒ in in human cells. *Biochemical and Biophysical Research Communications*, *411*(4), 762–767.

Oum, J.-H., Seong, C., Kwon, Y., Ji, J.-H., Sid, A., Ramakrishnan, S., Ira, G., Malkova, A., Sung, P., Lee, S. E., & Shim, E. Y. (2011). RSC Facilitates Rad59-Dependent Homologous Recombination between Sister Chromatids by Promoting Cohesin Loading at DNA Double-Strand Breaks. *Molecular and Cellular Biology*, *31*(19), 3924–3937.

Ouyang, Z., Zheng, G., Tomchick, D. R., Luo, X., & Yu, H. (2016). Structural Basis and IP6 Requirement for Pds5-Dependent Cohesin Dynamics. *Molecular Cell*, *62*(2), 1–12.

Panizza, S., Tanaka, T., Hochwagen, A., Eisenhaber, F., & Nasmyth, K. (2000). Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. *Current Biology : CB*, *10*(24), 1557–1564.

Parelho, V., Hadjur, S., Spivakov, M., Leleu, M., Sauer, S., Gregson, H. C., Jarmuz, A., Canzonetta, C., Webster, Z., Nesterova, T., Cobb, B. S., Yokomori, K., Dillon, N., Aragon, L., Fisher, A. G., & Merkenschlager, M. (2008). Cohesins Functionally Associate with CTCF on Mammalian Chromosome Arms. *Cell*, *132*(3), 422–433.

Pauli, A., Althoff, F., Oliveira, R. A., Heidmann, S., Schuldiner, O., Lehner, C. F., Dickson, B. J., & Nasmyth, K. (2008). Cell-Type-Specific TEV Protease Cleavage Reveals Cohesin Functions in Drosophila Neurons. *Developmental Cell*, *14*(2), 239–251.

Paull, T. T., Rogakou, E. P., Yamazaki, V., Kirchgessner, C. U., Gellert, M., & Bonner, W. M. (2000). A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Current Biology*, *10*(15), 886–895.

Paulovich, A. G., & Hartwell, L. H. (1995). A checkpoint regulates the rate of progression through S phase in S. cerevisiae in Response to DNA damage. *Cell*, *82*(5), 841–847.

Pepling, M. E. (2006). Decoding development in Xenopus tropicalis. *Genesis*, *44*(12), 622–633.

Peters, A. H. F. M., Plug, A. W., Vugt, M. J. van, & Boer, P. de. (1997). A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Research*, *5*(1), 66–71.

Potts, P. R., Porteus, M. H., & Yu, H. (2006). Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-

strand breaks. *The EMBO Journal*, *25*(14), 3377–3388.

Rankin, S. (2015). Complex elaboration: Making sense of meiotic cohesin dynamics. *FEBS Journal*, *282*, 2426–2443.

Rankin, S., Ayad, N. G., & Kirschner, M. W. (2005). Sororin, a substrate of the anaphase-promoting complex, is required for sister chromatid cohesion in vertebrates. *Molecular Cell*, *18*(2), 185–200.

Redon, C., Pilch, D. R., Rogakou, E. P., Orr, A. H., Lowndes, N. F., & Bonner, W. M. (2003). Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. *EMBO Reports*, *4*(7), 678–84.

Revenkova, E., Eijpe, M., Heyting, C., Gross, B., & Jessberger, R. (2001). Novel meiosis-specific isoform of mammalian SMC1. *Molecular and Cellular Biology*, *21*(20), 6984–6998.

Revenkova, E., Focarelli, M. L., Susani, L., Paulis, M., Bassi, M. T., Mannini, L., Frattini, A., Delia, D., Krantz, I., Vezzoni, P., Jessberger, R., & Musio, A. (2009). Cornelia de Lange syndrome mutations in SMC1A or SMC3 affect binding to DNA. *Human Molecular Genetics*, *18*(3), 418–427.

Rogakou, E. P., Boon, C., Redon, C., & Bonner, W. M. (1999). Megabase chromatin domains involved in DNA double-strand breaks in vivo. *The Journal of Cell Biology*, *146*(5), 905–16.

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., & Bonner, W. M. (1998). DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139. *The Journal of Biological Chemistry*, *273*(10), 5858–5868.

Rolef Ben-Shahar, T., Heeger, S., Lehane, C., East, P., Flynn, H., Skehel, M., & Uhlmann, F. (2008). Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. *Science*, *321*(2008), 563–566.

Rollins, R. A., Korom, M., Aulner, N., Martens, A., & Dorsett, D. (2004). Drosophila nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scc3 cohesion factor to facilitate long-range activation of the cut gene. *Molecular and Cellular Biology*, *24*(8), 3100–11.

Rollins, R. A., Morcillo, P., & Dorsett, D. (1999). Activation by Remote Enhancers in the cut and Ultrabithorax Genes. *Genetics*, *152*(2), 577–593.

Rowland, B. D., Roig, M. B., Nishino, T., Kurze, A., Uluocak, P., Mishra, A., Beckouët, F., Underwood, P., Metson, J., Imre, R., Mechtler, K., Katis, V. L., & Nasmyth, K. (2009). Building Sister Chromatid Cohesion: Smc3 Acetylation Counteracts an Antiestablishment Activity. *Molecular Cell*, *33*(6), 763–774.

Rubio, E. D., Reiss, D. J., Welcsh, P. L., Disteche, C. M., Filippova, G. N., Baliga, N. S., Aebersold, R., Ranish, J. A., & Krumm, A. (2008). CTCF physically links cohesin to chromatin. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(24), 8309–14.

Sanborn, A. L., Rao, S. S. P., Huang, S.-C., Durand, N. C., Huntley, M. H., Jewett, A. I., Bochkov, I. D., Chinnappan, D., Cutkosky, A., Li, J., Geeting, K. P., Gnirke, A., Melnikov, A., McKenna, D., Stamenova, E. K., Lander, E. S., & Aiden, E. L. (2015). Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proceedings of the National Academy of Sciences*, *112*(47), E6456–65.

Sartori, A. a, Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., & Jackson, S. P. (2007). Human CtIP promotes DNA end resection. *Nature*, *450*(7169), 509–514.

Schmitz, J., Watrin, E., Lénárt, P., Mechtler, K., & Peters, J. M. (2007). Sororin Is Required for Stable Binding of Cohesin to Chromatin and for Sister Chromatid Cohesion in Interphase. *Current Biology*, *17*(7), 630–636.

Seitan, V. C., Banks, P., Laval, S., Majid, N. a., Dorsett, D., Rana, A., Smith, J., Bateman, A., Krpic, S., Hostert, A., Rollins, R. a., Erdjument-Bromage, H., Tempst, P., Benard, C. Y., Hekimi, S., Newbury, S. F., & Strachan, T. (2006). Metazoan Scc4 homologs link sister chromatid cohesion to cell and axon migration guidance. *PLoS Biology*, *4*(8), 1411–1425.

Sherwood, R., Takahashi, T. S., & Jallepalli, P. V. (2010). Sister acts: Coordinating DNA replication and cohesion establishment. *Genes and Development*, *24*(24), 2723–2731.

Shibata, A., Conrad, S., Birraux, J., Geuting, V., Barton, O., Ismail, A., Kakarougkas, A., Meek, K., Taucher-Scholz, G., Löbrich, M., & Jeggo, P. a. (2011). Factors determining DNA double-strand break repair pathway choice in G2 phase. *The EMBO Journal*, *30*(6), 1079–1092.

Shinohara, A., Ogawa, H., & Ogawa, T. (1992). Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. *Cell*, *69*(3), 457–470.

Siede, W., Allen, J. B., Elledge, S. J., & Friedberg, E. C. (1996). The Saccharomyces cerevisiae MEC1 gene, which encodes a homolog of the human ATM gene product, is required for G1 arrest following radiation treatment. *Journal of Bacteriology*, *178*(19), 5841–5843.

Sjögren, C., & Nasmyth, K. (2001). Sister chromatid cohesion is required for postreplicative double-strand break repair in Saccharomyces cerevisiae. *Current Biology*, *11*(12), 991–995.

Skibbens, R. V., Corson, L. B., Koshland, D., & Hieter, P. (1999). Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes & Development*, *13*(3), 307–319.

Sonoda, E., Matsusaka, T., Morrison, C., Vagnarelli, P., Hoshi, O., Ushiki, T., Nojima, K., Fukagawa, T., Waizenegger, I. C., Peters, J. M., Earnshaw, W. C., & Takeda, S. (2001). Scc1/Rad21/Mcd1 Is Required for Sister Chromatid Cohesion and Kinetochore Function in Vertebrate Cells. *Developmental Cell*, *1*(6), 759–770.

Stedman, W., Kang, H., Lin, S., Kissil, J. L., Bartolomei, M. S., & Lieberman, P. M. (2008). Cohesins localize with CTCF at the KSHV latency control region and at cellular c-myc and H19/Igf2 insulators. *The EMBO Journal*, *27*(4), 654–66.

Stewart, G. S., Panier, S., Townsend, K., Al-Hakim, A. K., Kolas, N. K., Miller, E. S., Nakada, S., Ylanko, J., Olivarius, S., Mendez, M., Oldreive, C., Wildenhain, J., Tagliaferro, A., Pelletier, L., Taubenheim, N., Durandy, A., Byrd, P. J., … Durocher, D. (2009). The RIDDLE Syndrome Protein Mediates a Ubiquitin-Dependent Signaling Cascade at Sites of DNA Damage. *Cell*, *136*(3), 420–434.

Stiff, T., Driscoll, M. O., Rief, N., Iwabuchi, K., Lo, M., & Jeggo, P. A. (2004). ATM and DNA-PK Function Redundantly to Phosphorylate H2AX after Exposure to Ionizing Radiation. *Cancer Research*, *64*(7), 2390–2396.

Stigler, J., Çamdere, G., Koshland, D. E., & Greene, E. C. (2016). Single-Molecule Imaging Reveals a Collapsed Conformational State for DNA-Bound Cohesin. *Cell Reports*, *15*(5), 988–998.

Stokes, M. P., Rush, J., Macneill, J., Ren, J. M., Sprott, K., Nardone, J., Yang, V., Beausoleil, S. A., Gygi, S. P., Livingstone, M., Zhang, H., Polakiewicz, R. D., & Comb, M. J. (2007). Profiling of UV-induced ATM/ATR signaling pathways. *PNAS*, *104*(50), 19855–60.

Ström, L., Karlsson, C., Lindroos, H. B., Wedahl, S., Katou, Y., Shirahige, K., & Sjögren, C. (2007). Postreplicative formation of cohesion is required for repair and induced by a single DNA break. *Science*, *317*(5835), 242–245.

Ström, L., Lindroos, H. B., Shirahige, K., & Sjögren, C. (2004). Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. *Molecular Cell*, *16*(6), 1003–1015.

Sumara, I., Vorlaufer, E., Stukenberg, P. T., Kelm, O., Redemann, N., Nigg, E. A., & Peters, J. M. (2002). The dissociation of cohesin from chromosomes in prophase is regulated by polo-like kinase. *Molecular Cell*, *9*(3), 515–525.

Swagemakers, S. M. A., Essers, J., Wit, J. De, Hoeijmakers, J. H. J., & Kanaar, R. (1998). The Human Rad54 Recombinational DNA Repair Protein Is a Double-stranded DNA-dependent ATPase. *The Journal of Biological Chemistry*, *273*(43), 28292–28297.

Symington, L. S., Rothstein, R., & Lisby, M. (2014). Mechanisms and Regulation of Mitotic Recombination in Saccharomyces cerevisiae. *Genetics*, *198*(3), 795–835.

Takahashi, T. S., Basu, A., Bermudez, V., Hurwitz, J., & Walter, J. C. (2008). Cdc7-Drf1 kinase links chromosome cohesion to the initiation of DNA replication in Xenopus egg extracts. *Genes and Development*, *22*(14), 1894–1905.

Takahashi, T. S., Yiu, P., Chou, M. F., Gygi, S., & Walter, J. C. (2004). Recruitment of Xenopus Scc2 and cohesin to chromatin requires the pre-replication complex. *Nature Cell Biology*, *6*(10), 991–996.

Tang, J., Cho, N. W., Cui, G., Manion, E. M., & Shanbhag, N. M. (2013). Acetylation Limits 53BP1 Association with Damaged Chromatin to Promote Homologous Recombination. *Nat Struct Mol Biol*, *20*(3), 317–325.

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., Narita, T., Sixma, T. K., Choudhary, C., Bekker-Jensen, S., & Mailand, N. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature*, *527*(7578), 389–93.

Tonkin, E. T., Wang, T.-J., Lisgo, S., Bamshad, M. J., & Strachan, T. (2004). NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. *Nature Genetics*, *36*(6), 636–641.

Tóth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., & Nasmyth, K. (1999). Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes and Development*, *13*(3), 320–333.

Trujillo, K. M., Roh, D. H., Chen, L., Van Komen, S., Tomkinson, A., & Sung, P. (2003). Yeast xrs2 binds DNA and helps target rad50 and mre11 to DNA ends. *The Journal of Biological Chemistry*, *278*(49), 48957–48964.

Uhlmann, F., Lottspeich, F., & Nasmyth, K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature*, *400*(6739), 37–42.

Uhlmann, F., & Nasmyth, K. (1998). Cohesion between sister chromatids must be established during DNA replication. *Current Biology*, *8*(20), 1095–1101.

Ünal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J. E., & Koshland, D. (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Molecular Cell*, *16*, 991–1002.

Ünal, E., Heidinger-Pauli, J. M., & Koshland, D. (2007). DNA Double-Strand Breaks Trigger Genome-Wide Sister-Chromatid Cohesion Through Eco1 (Ctf7). *Science*, *1*(July), 245–249.

Vrouwe, M. G., Elghalbzouri-maghrani, E., Meijers, M., Schouten, P., Godthelp, B. C., Bhuiyan, Z. A., Redeker, E. J., Mannens, M. M., Mullenders, L. H. F., Pastink, A., & Darroudi, F. (2007). Increased DNA damage sensitivity of Cornelia de Lange syndrome cells : evidence for impaired recombinational repair. *Human Molecular Genetics*, *16*(12), 1478–1487.

Waizenegger, I. C., Hauf, S., Meinke, A., & Peters, J. M. (2000). Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell*, *103*, 399–410.

Watrin, E., & Peters, J.-M. (2009). The cohesin complex is required for the DNA damage-induced G2/M checkpoint in mammalian cells. *The EMBO Journal*, *28*(17), 2625–35.

Watrin, E., Schleiffer, A., Tanaka, K., Eisenhaber, F., Nasmyth, K., & Peters, J. M. (2006). Human Scc4 Is Required for Cohesin Binding to Chromatin, Sister-Chromatid Cohesion, and Mitotic Progression. *Current Biology*, *16*(9), 863–874.

Weber, S. A., Gerton, J. L., Polancic, J. E., DeRisi, J. L., Koshland, D., & Megee, P. C. (2004). The kinetochore is an enhancer of pericentric cohesin binding. *PLoS Biology*, *2*(9),

e260.

Weinert, T., & Hartwell, L. (1988). The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. *Science*, *15*(4863), 317–22.

Wendt, K. S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N., Maeshima, K., Shirahige, K., & Peters, J.-M. (2008). Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature*, *451*(7180), 796–801.

Wolner, B., & Peterson, C. L. (2005). ATP-dependent and ATP-independent roles for the Rad54 chromatin remodeling enzyme during recombinational repair of a DNA double strand break. *Journal of Biological Chemistry*, *280*(11), 10855–10860. http://doi.org/10.1074/jbc.M414388200

Wu, Z. J. (2009). A review of statistical methods for preprocessing oligonucleotide microarrays. *Statistical Methods in Medical Research*, *18*(6), 533–541.

Xu, B., Sowa, N., Cardenas, M. E., & Gerton, J. L. (2015). L-leucine partially rescues translational and developmental defects associated with zebrafish models of Cornelia de Lange syndrome. *Human Molecular Genetics*, *24*(6), 1540–1555.

Xu, H., Beasley, M. D., Warren, W. D., van der Horst, G. T. J., & McKay, M. J. (2005). Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. *Developmental Cell*, *8*(6), 949–961.

Yazdi, P. T., Wang, Y., Zhao, S., Patel, N., Lee, E. Y. H. P., & Qin, J. (2002). SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes and Development*, *16*(5), 571–582.

You, Z., Chahwan, C., Bailis, J., Hunter, T., & Russell, P. (2005). ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Molecular and Cellular Biology*, *25*(13), 5363–5379.

Zhang, J., Shi, X., Li, Y., Kim, B. J., Jia, J., Huang, Z., Yang, T., Fu, X., Jung, S. Y., Wang, Y., Zhang, P., Kim, S. T., Pan, X., & Qin, J. (2008). Acetylation of Smc3 by Eco1 Is Required for S Phase Sister Chromatid Cohesion in Both Human and Yeast. *Molecular Cell*, *31*(1), 143–151.

Zhang, N., Kuznetsov, S. G., Sharan, S. K., Li, K., Rao, P. H., & Pati, D. (2008). A handcuff

model for the cohesin complex. *Journal of Cell Biology*, *183*(6), 1019–1031.

Zhang, X., & Paull, T. T. (2005). The Mre11 / Rad50 / Xrs2 complex and non-homologous end-joining of incompatible ends in S . cerevisiae. *DNA Repair*, *4*(11), 1281–1294.

Zou, L., & Elledge, S. J. (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science*, *300*(5625), 1542–1548.


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